THE

American Journal of Physiology

VOL. XXXIV JUNE 1, 1914 NO. III

PARATHYROID DEFICIENCY AND SYMPATHETIC IRRITABILITY

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Received for publication April 2, 1914

IN the further development of the physiology of internal secretion a clean-cut knowledge of the relation of the various endosecretory organs to the sympathetic nervous system seems particularly desirable. In the hope of throwing additional light upon the subject a series of researches of which this is the third has been undertaken in this laboratory.[1]

There are to be found in the literature but few observations bearing upon the relation of the parathyroid glands to the sympathetic system. Falta and Kahn[2] in 1912 studied the effects of parathyroid extirpation in a dog. Both parathyroids were removed on one side leaving the thyroid lobe. On the other side the thyroid lobe with the two parathyroids was removed. Later blood pressure was recorded and the reaction to epinephrin in various dilutions determined. A *depression* was observed with quantities of the drug which in the normal animal give a *pressor* effect. The significance of the observation is not clear. It is interpreted by Falta and Kahn as indicating an increased sympathetic irritability. In view of the fact, however, that the first reaction of a dog to

[1] Hoskins and Wheelon: This Journal, 1914, xxxiii, pp. 81, 172.

[2] Falta and Kahn: Zeitschrift für klinische Medicin, 1912, lxxiv, p. 108.

ascending dosages of epinephrin is depression and that this reaction was relatively late in appearing, their results would seem rather to indicate a lowering of sympathetic irritability following parathyroidectomy. In the same article Falta and Kahn report also the results of a series of studies of cases of clinical tetany. In these the patients characteristically showed an augmented irritability to epinephrin as judged by increase of blood pressure or pulse rate. In the absence of definite proof that the tetanies observed were due to parathyroid deficiency their findings are not conclusive as regards the subject of this paper. An increased sensitiveness to pilocarpin was also observed. It was concluded, therefore, that in parathyroid deficiency there is an augmented irritability of the whole autonomic system.

Lately there have been published from Carlson's laboratory several papers which deal with parathyroid deficiency. In 1912 Carlson [1] concluded from a study of the activities of the digestive tract that parathyroid extirpation causes both in the cat and in the dog a depression of the sympathetic system. Although in the final stages of tetany in his cats a marked depression of the motor function of the stomach and intestines was observed, in most instances the animals in this respect were normal. In dogs, however, both the motor and secretory activities deviated in the direction of depression. On the face of it the evidence would seem to indicate an overactivity of the splanchnic sympathetics, but Carlson interprets it otherwise. Salivation was noted as a common symptom, but this was ascribed to bulbar autonomic rather than sympathetic influences. Other observations were recorded upon mydriasis, sweating, bladder tonus, defecation and parturition, some of which suggest abnormal sympathetic activity, but which as a whole were regarded as indicating sympathetic depression. In a later paper Carlson [2] records that during attacks of tetany after thyro-parathyroidectomy there is a marked loss of gastric muscular tonicity; during the interim between attacks the tonus returns to normal. Similarly Keeton [3] has found a depression of gastric secretion, a decreased hydrogen ion and probably decreased pepsin out-

[1] CARLSON: This Journal, 1912, xxx, p. 309.
[2] CARLSON: *Ibid.*, 1913, xxxii, p. 398.
[3] KEITON: *Ibid.*, 1914, xxxiii, p. 25

put which appears as parathyroid tetany develops. Stoland [1] has noted a depression of bile and of pancreatic juice under similar conditions. These observations, all of which suggest excessive sympathetic functioning, are interpreted in harmony with Carlson's first conclusions as due to other causes.

In our experiments blood pressure was used exclusively as a criterion of sympathetic conditions. The comparative irritability of the sympathetic system before and after parathyroid destruction was determined by injecting fixed quantities of nicotin and of "adrenalin." To test the condition of the muscular tissues involved pituitrin was also used in several cases. In all the experiments dogs were employed. The technique in general was the same as that of the preceding investigations of the series. The animals were anesthetized with ether. Then with aseptic precautions a reservoir cannula was inserted into a femoral artery for taking blood-pressure records and a simple large-bore cannula into the corresponding vein for the injecting of fluids. The standard doses of drugs employed were usually adrenalin 0.5 c.c. and 1.0 c.c. 1:50,000 dilution, nicotin 0.5 c.c. and 1.0 c.c. 1:2,000 and pituitrin 0.1 c.c. In case an animal was small or showed unusual irritability the dosage was correspondingly diminished. With a view to its subsequent use the reaction to 1 gm. of calcium lactate given intravenously was also determined. The condition of the vasomotor system having been established, the opened blood vessels were tied off and the incision closed and sutured.

Immediately preceding or following the blood-pressure determinations the thyroid glands were exposed by bringing them out through a median incision. An attempt was then made to identify all four parathryoid glands. In several cases the attempts were successful. All the glands found were destroyed by cautery. In case both glands were found on one side only, the thyroid of the opposite side was removed with its capsule so as to include both parathyroids. In other instances where only posterior parathyroids were found these were cauterized and the anterior half of each thyroid removed so as to include the anterior parathyroids. Asher and Rodt [2] have concluded that thyroid secretion exerts a considerable

[1] Stoland: *Ibid.*, 1914, xxxiii, p. 283.

[2] Asher und Rodt: Centralblatt für Physiologie, 1912, xxvi, p. 223.

effect upon the sympathetic system. In nearly all cases, therefore, care was taken to leave sufficient thyroid tissue for normal functioning, but in two animals complete removal of all demonstrable thyroid and parathyroid tissue was made. Aseptic technique of course was employed. The incisions were closed and the animals kept from one to five days.

Blood pressures and the reactions to "adrenalin," nicotin, and pituitrin were then taken with the same dosages as before, using the blood vessels of the other hind leg corresponding to those used in the first determinations. In case a third determination was made the cannulas were inserted in one or the other of the femoral vessels proximal to the former incision. In several instances 1 gm. of calcium lactate was given intravenously and the drug injections repeated.

In only one case (No. 62) in which the animals survived (aside from one nearly moribund of hemorrhage) did we fail to get clear evidence of parathyroid deficiency. Nearly all the animals developed pronounced tetany. In one, however, the only evident sign was an augmentation of reflexes. A light tap on the paw or hip would result in a quick jerk of the leg. Otherwise the dog showed a disinclination to move. In the case of the animal (No. 62) in which no signs of parathyroid deficiency were detected an apparently normal gland was found at autopsy; this therefore is not a valid negative case. Of all the animals showing external evidence of deficiency, only one failed to show also an augmented sympathetic irritability. The blood-pressure determination was made in this case 24 hours after the first operation. A later determination was not made. So far as it goes this observation indicates that augmentation of irritability develops in the sympathetic system more slowly than in the voluntary neuromuscular apparatus.

Figure 1 shows the results secured with adrenalin in case of dog No. 60. (*a*) shows the reaction to 0.5 c.c., 1:50,000. The parathyroids were then removed. (*b*) shows the reaction to the same quantity of adrenalin two days later. One gram of calcium lactate was given intravenously and a few minutes later the reaction (*c*) again determined.

Figure 2 (*a*) shows the effect in dog No. 55 of 0.5 c.c. of nicotin, 1:2000, before the operation and 2 (*b*) the effect of the same dosage two days after parathyroidectomy.

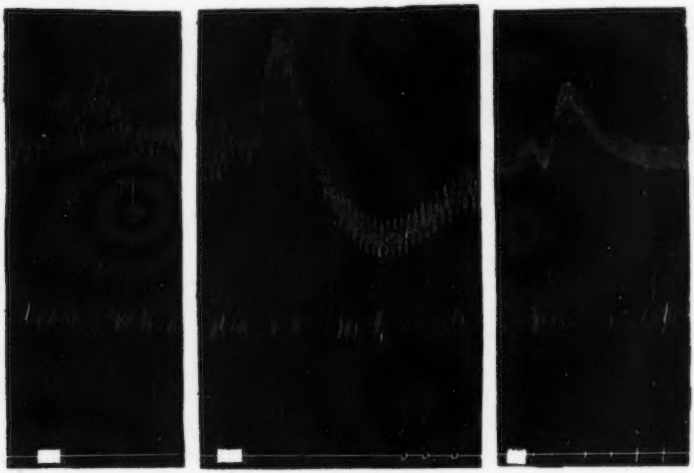

FIGURE 1. (*a*) Reaction in normal dog to 0.5 cc. "adrenalin," 1:50,000. (*b*) Reaction to same dosage in same dog 2 days after parathyroid destruction. (*c*) Reaction to same dosage after 1 gm. calcium lactate intravenously. Blood pressure from femoral artery.

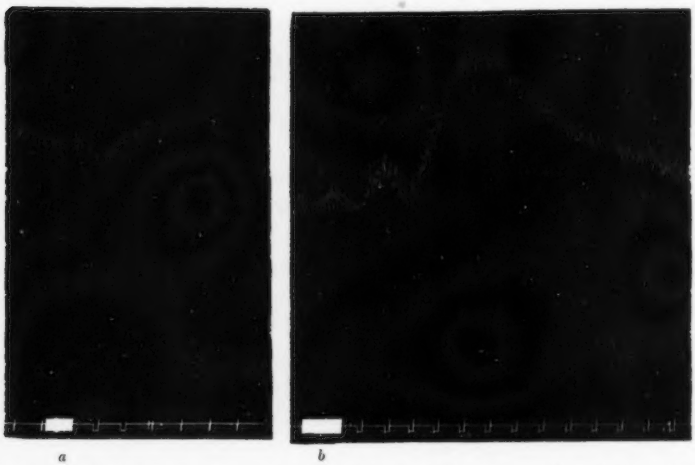

FIGURE 2. (*a*) Reaction in normal dog to 0.5 cc. Nicotin 1:2,000. (*b*) Reaction in same dog to same dosage 2 days later, after destruction of parathyroid glands. Blood pressure from femoral artery. Time 5 sec.

Dog No. 68 exemplified the trend of the investigation as a whole. The protocol is as follows:

Old female coach dog. Wt. 8.9 Kilos.

March 19, 1914, 9:50 A.M. Animal etherized. Cannulas inserted into right femoral artery and vein.

10:15–10:35 Reactions determined to nicotin 0.3 and 0.5 c.c. 1-2000, adrenalin 0.5 c.c., 1:50,000 (5-minute intervals).

10:40 Injected calcium lactate 1 gram.

10:50–11 Reactions to nicotin 0.5 c.c. 1:2000, adrenalin 0.5 c.c. 1:50,000. Reactions slightly greater than before calcium.

11:10–11:40 Four parathyroids demonstrated and cauterized. Thyroids slightly larger than normal, parathyroids apparently normal.

March 21, 10 A.M. Clonic convulsions observed. Animal otherwise appeared normal.

12:00 M. Fine tremors throughout body but especially in limbs. No clonus.

1:30 P.M. Condition same as at noon.

1:50 Etherized. Fine tremors persist. No clonus. Animal vomited during operation of inserting cannulas.

2:06–2:20 Reactions to nicotin and adrenalin as before. Reactions slightly increased above normal.

March 23, 2:30 P.M. Fine tremors.

March 24, 10:00 A.M. Clonic convulsions and tremors throughout body.

1–2:30 P.M. Slight tremor, particularly in shoulder muscles. No clonus.

2:30–3:50 Occasional clonus of different muscles, gradually becoming more frequent. Reflexes in legs (mechanical stimulus) augmented.

3:50 Etherized. Cannulas inserted, femoral vessels.

4:12–4:25 Reactions to nicotin 0.3 c.c., 0.5 c.c., adrenal 0.5 c.c. as before. Reaction to nicotine markedly increased. To adrenalin very slightly decreased.

4:26 Calcium lactate 1 gram.

4:30–4:55 Nicotin 0.5 c.c. at 5-minute intervals. Reaction gradually decreased to 4:45 then increased nearly to previous level.

Animal killed.

Autopsy: Thyroids normal. No parathyroid tissue discoverable. Scars of four cauterizations.

In view of Carlson's observation that loss of gastric tonicity occurs only during attacks of frank tetany with return approximately to normal in the interim we rather expected to find a parallelism between the severity of external symptoms and the degree of vasomotor irritability. The researches as a whole, as in case of No. 68, have given the impression that to some extent this is true, but various exceptions were noted. No. 55, for example, showed as great augmentation of vasomotor irritability at the third determination when there was complete absence of clonus as in the second when clonus was severe. No. 63, 24 hours after operation, showed pronounced clonus and tremor with no augmentation of vasomotor irritability, while No. 65 in an interval of freedom from clonus and tremors after an attack of severe tetany showed augmented sympathetic irritability.

The reactions to pituitrin were in general augmented at the same time as those to nicotin and adrenalin, but usually not to the same degree; in some instances they were greater, in some less. As a whole, however, they indicate that the vascular musculature itself shares to some extent in the general heightened irritability.

Similarly there was a lack of consistent parallelism between the reactions to nicotin and to adrenalin. Sometimes one, sometimes the other was greater. A comparison of the reactions to all three drugs in various cases indicates that parathyroid deficiency affects all three components of the vasomotor apparatus, — the sympathetic ganglion cells, the myoneural junctions and the musculature itself.

Keeton found that calcium injections reduce the gastric symptoms of parathyroid deficiency as they do the tetany. In case of the vasomotor symptoms this also seems to some extent to be true. A considerable experimental difficulty in determining the matter is that calcium affects the vasomotor mechanism in the normal animal causing a slower, stronger heart beat and sometimes an increased, sometimes a decreased irritability to the stimuli used.

Dog 68 showed the clearest evidence of a sedative effect of calcium. In the normal condition the animal showed augmented vasomotor irritability after 1 gram of calcium lactate given intravenously. After parathyroidectomy there was a gradual reduction of irritability for fifteen or twenty minutes after the injection of

a similar dose of calcium. After twenty minutes, however, the irritability again began to increase and was back almost to the original point at the end of a half hour.

We were able incidentally to confirm Carlson's observation that salivation is a common result of parathyroid deficiency.

No difference was observed in the results of parathyroidectomy alone and the removal of both the thyroids and parathyroids.

A possible source of error in such investigations as the foregoing is the spontaneous changes of vasomotor irritability under the conditions of our experiments. Several months' use of the technique, however, has given an appreciation of its limitations and we have no hesitancy in affirming that the increased irritability observed after parathyroid destruction is decidedly greater than the variability which occurs in normal animals. Moreover, the variability is all in one direction, whereas, in normal animals, it is of course either way, at random.

Summary and Conclusions

1. Parathyroid destruction in dogs results in a marked increase of vasomotor irritability as shown by the reactions to nicotin, epinephrin and pituitrin.

2. All components of the vasomotor mechanism, sympathetic cells, myoneural junctions and musculature, seem to be affected. The effects are of varying degree in different cases.

3. There was observed no strict parallelism between the external symptoms of parathyroid deficiency and the degree of vasomotor irritability.

4. Inconclusive evidence indicates that calcium injections in some measure restore vasomotor irritability toward normal.

5. The sympathetic system offers no exception to the general increase of irritability that results from parathyroid extirpation.

THE CONTENT OF SUGAR IN THE BLOOD UNDER COMMON LABORATORY CONDITIONS

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Received for publication April 18, 1914

I. — Introduction

THE use of variations in the concentration of sugar in the blood as an indication of the response of the animal to experimental conditions offers many theoretical advantages over the use of the presence of, or variations in the amount of, sugar in the urine. This is true, first, because changes in either direction may be detected. While sugar is always present in the blood, it is ordinarily present in the urine in minimal quantities only. The urine, therefore, can ordinarily be used to show only an increase in mobilized sugar, while the blood will show either an increase or a decrease. Secondly, profound changes of concentration of sugar may occur in the blood without giving rise to a detectable glycosuria. This may be due to the short duration of the change, or the change may not be of sufficient magnitude to lead to the excretion of sugar by the kidneys or it may possibly be due to a modification of the kidneys themselves. This very sensitiveness may, however, lead to serious difficulties in the handling of the animals before and during any experiment which involves the estimation of sugar in the blood. The third and most fundamental advantage lies in the intimate relation which exists, on the one hand, between the blood and the cells which are using the sugar and, on the other, between the blood and the stores of carbohydrate.

Presumably it is the greater difficulty of technique which has deterred many investigators from using glycaemia rather than glycosuria, as the criterion of change in the organism. Too often this has detracted greatly from the value of the research. Others have recognized the more fundamental bearing of variations in the

sugar of the blood but apparently without recognizing the great delicacy of the mechanism with which they were working. The result is that in many cases well-conceived experiments are largely vitiated by inadequate or improper controls. Hirsch and Reinbach and Rolly and Opperman [1] have recently called attention to the necessity of controlling, as far as possible, every factor in experiments of this nature.

The purpose of this paper is threefold: first, to determine, if possible, a set of conditions under which the amount of sugar in the blood of one laboratory animal, the cat, will be approximately constant. Upon such conditions, when once established, experimental conditions may be superimposed with reasonable assurance that differences from the constant are due to the new factors; secondly, to study the effects of some of the common conditions to which animals are subjected before being submitted to the experimental procedures in order that those which modify the concentration of sugar in the blood may be determined; and, lastly, to study, for the same reason, a few of the experimental procedures frequently used in experiments involving the estimation of the sugar in the blood. Abundant evidence from the literature, as well as my own work, shows that the most painstaking attention to all details is demanded if trustworthy results are to be obtained.

Although, as will be seen from the above, the experiments to be reported were not primarily planned to throw light upon the problems of the mobilization and use of sugar by the organism, it is thought that some of the results found may have a deep physiological significance. An extended discussion of the theoretical or possible significance of my results would, however, be out of place at this time. The attempt is made to discover and catalogue a few of those disturbing factors which are constantly entering into our experiments, unbidden and frequently without our knowledge, and which lead us to false conclusions. Many, perhaps most, of the factors studied by me have been previously investigated for other animals and indeed some of them for the cat. It was never-

[1] References to the literature cited will be found in Section VII, arranged alphabetically according to authors. Where more than one article is cited from one author the particular articles to which reference is made is indicated by the small numbers.

theless thought desirable to correlate the results for a single animal and by a single uniform method. In part because there has been comparatively little work done upon the cat, and in part for the reasons given below, this animal was selected for the research.

Long ago Boehm and Hoffman called attention to some of the advantages of cats for laboratory work. They mentioned especially their cleanly habits, their uniform size and the fact that they had found them to be more uniformly healthy than other common animals available for estimation of the sugar of the blood. There are, however, other and perhaps more fundamental advantages. A large amount of work has been done on excised muscles — a type of experiment for which the cat seems to be particularly adapted. Notes published by Lee and by Lee and Harrold show that some of this is directly related to the use of sugar by the organism. Again, some authors, as Macleod and Pearce, have sought to avoid, by decerebration, the extended use of drugs in experiments where prolonged anesthesia is necessary. The same results may be obtained in a more physiological manner, and with less hemorrhage, by cerebral anaemia. Leonard Hill and Stewart with his co-workers have shown that the dog, because of peculiarities of the blood-supply to the brain, is not so well adapted for this procedure as is the cat. Pike has confirmed Porter's statements that cats are better adapted for experiments involving vasomotor responses than are dogs.

II. — Method of Analysis

The preparation of the animal and method of obtaining the blood will be discussed later. Only the chemical processes involved will be described here. It is not possible to estimate the sugar by any known method in the presence of protein. Many reagents have been used and many methods proposed for the removal of the protein from blood preparatory to the determination of sugar. In 1908 Michaelis and Rona [1] proposed the use of colloidal iron hydroxide for this purpose. Their method has been well received and is widely used at present. Recently, however, Lesser reports that it is not satisfactory, in the form proposed by the authors for the blood of either frogs or turtles. In the

limited use that I have made of the method I have found it fairly satisfactory but have preferred the phosphotungstic acid method described below. A method which requires but a small amount of blood for the analysis possesses many obvious advantages. Because of this the method recently described by Lewis and Benedict or the micro-chemical methods of Bang or of Michaelis will prove of great value to both the clinician and the experimentalist, provided that they give the same satisfactory results in other hands that have been reported for them by their authors. Dehn and Hartman are now publishing a series of researches in which they are developing a method for the use of picric acid as the oxidizing agent in sugar determinations. Because of the greater delicacy claimed for it the picric acid method may supplant the use of copper for this purpose. The method which I have used is very similar to the one used by Pfeffer for the removal of proteins from bacterial cultures prior to the determination of sugar. Reid and, more recently, Oppler have described methods for removing the protein from blood by this reagent.

In my own method the blood was drawn directly from the blood-vessels into a beaker weighed with sufficient 1 per cent ammonium oxalate to make the final concentration of oxalate in the blood about 0.25 per cent. The beaker was constantly shaken while the blood was being drawn. The second weighing was made at once. Any blood on the sides of the beaker was then washed down with distilled water and about 300 cc. of water added. This was done for the double purpose of preventing glycolysis, as suggested by Rona and Döblin, and of breaking down the corpuscles so that any sugar contained within them might be freed. This was suggested by the work of A. Loeb, Rona and Michaelis,[2] Rona and Takahashi and others. As soon as the laking appeared complete the solution was washed into a 500 cc. volumetric flask, which was then filled to the mark with distilled water. It was then divided into two equal portions with the aid of a 250 cc. flask, and each portion was washed into a precipitation jar. About 1.2 cc. of a freshly prepared 10 per cent solution of phosphotungstic acid was then added for each gram of blood taken. This addition was made slowly from a dropping funnel while the mixture was being stirred with a mechanical stirrer. About twenty minutes was allowed for this precipita-

tion, but, thanks to the dropping funnels and the stirrers, did not consume much time on the part of the operator. The result was a brown or chocolate colored precipitate, from which a limpid filtrate rapidly separated, that gave none of the common protein reactions. After the precipitation was complete, each portion was washed into a 500 cc. flask, which was then filled to the mark, and filtered through an ordinary filter without suction. An aliquot part of about 350 cc. was taken for analysis.

The phosphotungstic acid was removed by the addition of 25 cc. of a saturated solution of barium hydroxide. After this addition, the mixture was allowed to stand for a time at room temperature. It must not be heated at this point, nor should it be allowed to stand much longer than is necessary to complete the reaction. The completion of the reaction was determined by the addition of a few drops of the barium solution to a few cubic centimeters of the clear supernatant fluid. When the reaction was complete it was again filtered and the precipitate was well washed with water. This filtrate was rendered just acid to litmus with sulphuric acid to precipitate the excess of barium, and the barium sulphate was removed by filtration. The final filtrate was evaporated to about 50 cc. in a Jena evaporating dish, and the sugar was estimated by the "Uniform method for sugar analysis" described by Munsen and Walker. Calculations were made from the tables given in Bulletin 107, Edition of 1912, of the Bureau of Chemistry, United States Department of Agriculture. The Bulletin, in addition to the table, contains a brief description of the method.

The accurate control of the estimation of sugar in blood or other solutions containing protein is very difficult. The present method was controlled as follows. A sample of blood was prepared as described above, except that a known quantity of glucose was added to one of the two portions just before the precipitation was begun. From this time on the estimation was completed in the usual manner. Evidently the amount of sugar recovered from the portion to which the addition was made, less the amount added, should be equal to that recovered from the other portion. Reference to Table 1 will show that this was the case within the limits of error permissible for work of this type. This method presupposes that the added sugar exists in the blood in the same condition as

TABLE 1

To show Degree of Recovery of Sugar. The Blood in Experiment 6 was Divided into Four Portions: *a*, *b*, *c* and *d*. Sugar was Added to Portions *c* and *d* as Indicated. In Experiment 17 the Blood was Divided into Three Portions and Sugar Added only to *c*. Experiment 20 was Carried out just as Described in the Text.

	Experiment No. 6				Experiment No. 17			Experiment No. 20	
	a	b	c	d	a	b	c	a	b
			Glucose added	Glucose added			Glucose added		Glucose added
Gm. blood in sample	47.51	47.51	47.51	47.51	49.41	49.41	49.41	50.67	50.67
Total glucose recovered	0.0314	0.0328	0.0501	0.0497	0.0334	0.0339	0.0514	0.0358	0.0538
Less glucose added	—	—	0.0178	0.0178	—	—	0.0195	—	0.0195
Blood sugar recovered	0.0314	0.0328	0.0323	0.0319	0.0334	0.0339	0.0319	0.0358	0.0343
Blood sugar gm. per cent	0.0661	0.0690	0.0680	0.0671	0.0676	0.0686	0.0646	0.0705	0.0677
Av. of similar samples	av. a + b	0.0676	av. b + c	0.0676	av. a + b	0.0681	0.0646	—	—

that naturally present — a presumption which is by no means proved. For this reason, even though the results are satisfactory, one cannot be sure that all of the sugar has been recovered.

The degree to which results obtained by any one method are consistent, one with another, gives another means of judging of the accuracy of the method. The reader will have to be the judge of the way in which the present method responds to this test after having studied the tables submitted — especially Table 5.

Rona and Michaelis [1] have compared different methods for the removal of protein from blood and have found a variation in the amount of sugar recovered after the use of the several methods. This, they believe, is because the glucose does not all exist in the blood in simple solution. There is no present need for postulating the exact condition of the sugar, since any aggregation of the carbohydrate molecules or any combination of them with either protein or lipoid might easily interfere with the complete recovery of the dextrose by any of the methods available.

Some authors, notably Arthus, and Rosenfeld and Asher, have sought to show, by dialysis, that the sugar exists in the blood in simple solution. Consideration of the law of mass action, however, reveals the limitations of this method of attack. The equilibrium is at once destroyed by the removal of any portion of the sugar which may be in true solution. The disturbed condition will bring about a continuous dissolution of any loose combinations present so long as the removal occurs. In this way it is conceivable that a great deal of sugar may be removed by dialysis which did not originally exist in free solution.

From these considerations it follows that before the work of different authors or the results obtained by different methods may be compared, a factor of comparison must be established. That is, the amount of sugar recoverable from the same blood by each of the methods, under the same conditions, must be found, and the resulting ratios considered in making comparisons.

If it is true that the form in which the sugar is present in the blood influences the amount recoverable by the different methods, it follows that the amount recoverable by one and the same method may be expected to vary with the variation of the condition of the sugar or of any of its combinations which occurs as a result of

experimental procedures. Thus, the difficulty of interpretation of results all of which are obtained by the same method is also increased. We have at present no means of knowing that the sugar exists in the blood in the same state under different experimental conditions to which the animal has been exposed. Thus a rise or fall in recoverable sugar following any experimental change to which the animal may have been subjected, may be due to a change in the condition of the sugar in the blood with no variation in the absolute amount.

These possibilities of misinterpretation must be kept in mind in studying the following results.

III. — The Effect of Some of the Preliminary Conditions upon the Concentration of Sugar in the Blood

Under this head will come only those factors which, apart from the actual experimental conditions, interfere with the concentration of sugar.

The changes in environment undergone by an animal on entering the laboratory cannot be presumed to be without influence upon the point in question. Hence, if uniform results are to be expected, sufficient time must be allowed for all of the animals to establish themselves in equilibrium with their new surroundings. Of the many factors that might play a part in bringing about variations, two seemed especially liable to do this. These were, first, the changes in the character of the diet and feeding habits, and, secondly, the mental excitement incident to the new conditions. Time must be allowed the animals to establish themselves upon their new diet and to become accustomed to their new environment. Of the two, very probably the latter is the more productive of variations. A week seemed none too long a time to allow to the animals for this purpose, and hence was taken as the minimum limit in the usual routine. The few animals which were killed after a shorter period in the laboratory will be specially mentioned in the tables.

In Table 2 it is shown that the physical condition may be a disturbing factor. Here it is seen that the concentration of sugar may be high, as in numbers 58, 74 and 106, or be in essential

TABLE 2

TO SHOW THAT THE CONCENTRATION OF SUGAR IN THE BLOOD MAY BE DISTURBED BY THE ABNORMAL PHYSICAL CONDITION OF THE ANIMAL

No. of Exp.	Sex	Body wt. k.	Blood drawn gm.	Blood per k. body wt. gm.	Gm. % glucose recovered	Var. from stand. mean—Table 5		Values calculated to 30 gm. blood per k. body wt.		Remarks
						Absolute variations	In % of stand. mean	Calculated concentration	% of var. from stand. mean	
31	—	—	126.88	—	0.075	+ 0.006	9	—	—	Abscess in jaw
58	M	2.99	107.35	35.90	0.107	+ 0.038	55	0.111	+ 61	Severe respiratory infection
60	M	2.41	75.40	31.28	0.062	- 0.007	10	0.063	- 9	Late recovery from respiratory inf.
61	M	2.92	72.91	24.93	0.068	- 0.001	1	0.064	- 7	Respiratory inf. early stages
74	M	2.50	59.43	23.77	0.086	+ 0.017	25	0.082	+ 19	Emaciated, cause unknown
95	M	2.35	79.69	33.91	0.047	- 0.022	32	0.050	- 28	Emaciated, unkempt, long standing respiratory inf.
106	M	3.12	88.80	28.46	0.101	+ 0.032	46	0.100	+ 45	Localized abscess on head

harmony with that of normal animals as shown by numbers 60 and 61; again in one case, number 95, which had apparently been running for a long time, the concentration was low. From this it is evident that one of the conditions for concordant results is the rigid exclusion of all animals which are not, so far as can be determined, in good health. While, as was shown above, a certain minimal stay in the laboratory should be allowed all animals before the sample is taken, too long a preparatory period is not desirable. The animals in general do not do as well in confinement as when free, and become especially liable to infection. For this reason, too, in experiments of long duration great care must be taken to protect the animal from all forms of infection and other influences which, aside from the purely experimental conditions, might lead to a changed physical condition.

The length of the period intervening between the last feeding and the collection of the blood may be an important factor. Bang and others have fed animals varying amounts of different carbohydrates in solution, and have followed the resulting changes in the concentration of sugar in the blood. Böe agrees with Bang that the hyperglycaemia induced in rabbits by this means has disappeared by the end of the third hour. Fischer and Wishart report a return to normal, in dogs which have ingested fifty grams of glucose in solution, by the end of the second hour. There is no doubt but that such experiments are of great value in determining the changes in glycaemia which take place under the conditions of the experiment. However, conclusions as to the conditions following an ordinary meal must be drawn with caution, since the time relations following the ingestion of protein and fat, or of these with starch, are not necessarily the same. This is true not only because of the different quantities of carbohydrate taken into the body under the different conditions, but also because of the difference in rates of absorption dependent upon the necessity for digestion in the usual meal and the interference arising from the other elements of the meal. In any case, it was thought best to allow sufficient time for any passing disturbance to disappear. With two exceptions the animals were allowed to live from sixteen to twenty-four hours after the last meal before the sample of blood was taken. In a number of cases the alimentary canals were

examined and found empty as far as the ileocoecal valve. Each of the two exceptions noted above were killed three hours after a meal, one of meat, the other of bread and meat. The one which had received meat alone, number 77, yielded 0.066 per cent sugar, which is, as will be seen by comparing with Table 5, in approximate agreement with the standard. The other cat, number 76, yielded a concentration of 0.086 per cent. This should, of course, be compared with the results shown in Table 4. When this is done it is seen that it is well within the limits of variation. Hence no significance can be attached to the variation in a single experiment from the average — 0.078 per cent — which is found for the corresponding series.

There seems to be some difference of opinion with regard to the effect of the character of the diet. Seelig finds less disturbance of the concentration of sugar in the blood of dogs given ether when the diet has consisted of bread for several days than when it has consisted largely of meat. As this point is of so much importance to the experimentalist, some attention was given to it. A diet consisting only of bread was found to be impractical for cats, so that they were given stale bread and cooked beef hearts, approximately pound for pound, together with the water in which the hearts were cooked. Even on this diet the animals did not do so well and were more subject to respiratory infection than those receiving the diet to be described later. It was not usually possible to keep them in a satisfactory condition on this diet for a longer time than two weeks. Aside from this, or perhaps because of it, a constancy of results for the quantity of sugar in the blood could not be obtained which approached that with the other diet. The results obtained are summarized in Table 4. It will be noticed that the variation between the extremes — 0.056 per cent and 0.104 per cent — is equal to 86 per cent of the smaller number and that 83 per cent vary from the average of the series by more than 10 per cent. Evidently this is not a satisfactory diet where a constant concentration of sugar is the end sought.

The other diet was cooked beef hearts with the bread omitted. This was found to be more satisfactory. While, as seen from Table 5, the extreme variation is between 0.096 per cent and 0.056 per cent and is thus almost as great as the variation of the previous

series, only 25 per cent of the animals vary from the mean by more than 10 per cent. Cf. Table 9.

Why the average for the animals allowed carbohydrate food in addition to the meat should be higher than that for those given meat alone, is a question difficult of answer. According to the ideas generally held, the character of the food is immaterial beyond the first few hours after the meal. Further, if the difference is due directly to the differences of diet, one would not look for the extreme variations which were found. There are other possibilities, however. Reference to Table 2 shows that animals with some types of infection seem to have a relatively higher content of sugar than the standard animals. As above noted, animals fed on the bread and meat diet are more prone to infection, and it is possible that in some cases incipient disease was overlooked. Again these animals were more restless and quarrelsome than those on the meat diet, and this would tend toward higher results. Rose thinks that carbohydrate feeding does not materially increase the amount of sugar in the blood of rabbits. It is, though, well to note in this connection that the rabbit is a herbivore, and as such may have better provision for handling carbohydrates than the cat, which is by nature a strict carnivore. One experiment reported by Rolly and Opperman [3] indicates that it is immaterial whether the protein given to the dog is derived from animal or vegetable sources. Jacobsen's [2] results are also of interest here.

It has long been known that the more intense emotions are a frequent cause of glycosuria. This was early spoken of by Rayer and somewhat later by Frerichs. Recently Cannon and some of his co-workers have laid especial stress upon this form of glycosuria; and have shown that for cats at least it is of purely psychological origin. Pavy [1] speaks of the necessity for "tranquillity" on the part of the animal while the sample is being drawn, and Eckhard emphasizes the fact that rabbits must not be tied in the holder for work involving glycosuria. Naunym very early reported an increased amount of sugar in the blood of animals which had been bound. Among the later writers Jacobsen,[1] Hirsch and Reinbach and Loewy and Rosenberg [1] have discussed in detail many of the difficulties in the way of the use of rabbits for experiments of this type. Presumably this difficulty lies, in large part at

least, in the nervous disposition of these animals and their proneness to excitement. Rolly and Opperman[3, 5] discard them as entirely unsuited for such work. Seelig finds no glycosuria in one dog which had been bound for two and one-half hours. Rolly and Opperman[3] also think that dogs may be safely used for such experiments, while Loewy and Rosenberg on the other hand find the concentration of sugar in the blood of both dogs and of rabbits increased by sensory stimulation, though, it is true, the increase in the dogs was not so marked.

Boehm and Hoffmann first demonstrated glycosuria in cats as a result of binding them on a holder and so called it "Fesselung Diabetes." This result has been interpreted as being due to various factors, as mental excitement, loss of heat, and muscular exertion. It has been shown in Cannon's laboratory that the first factor alone is sufficient. He therefore suggests the term "emotional glycosuria." The general fact that hyperglycaemia and frequently glycosuria follow excitement in all laboratory animals, with the possible exception of the dog, and in man is widely accepted. The only reason for adding to the already extended literature of the subject is to find to what extent the handling of an animal which is necessary for obtaining a sample of blood or in preparing it for an experiment may disturb the standard conditions. In my experiments, all animals in which excitement was evident were discarded, except as noted in the tables. A few in which excitement was evident were killed, and the results proved the necessity of Pavy's rule of complete tranquillity if consistent findings are desired. Two animals, numbers 108 and 110, were held, as if given ether by a cone, though ether was not actually given in either case. A third was placed in a bell jar for about the length of time that would have been required for etherization, had ether been given. Others were subjected to other conditions which are apt to occur in the laboratory and which produced slight excitement, as indicated by crying or otherwise. The results, with a brief description of the conditions in each case, are given in Table 3. It will be seen that in every case there is a noticeable rise in the amount of sugar contained in the blood. These results show that the animal must be, as Pavy says, tranquil, not alone at the time that the sample is drawn, but for some time before. From

TABLE 3

To Show that the Ordinary Handling to which Animals are Subjected in the Course of an Experiment may Cause a High Concentration of Sugar in the Blood.

No. of Exp.	Sex	Body wt. k.	Blood drawn gm.	Blood per k. body wt. gm.	Gm. % glucose recovered	Var. from stand. mean-Table 5		Gm. % calculated to 30 gm. blood per k. body wt.		Remarks
						Absolute	% of mean	Concentration	Variation in % of stand. mean	
51	F	2.60	75.57	29.06	0.102	+ 0.033	48	0.101	46	Nervous in laboratory before killing
52	M	3.50	107.65	30.76	0.078	+ 0.009	13	0.078	13	In bag 1¾ hrs. before killing, quiet
64	M	2.11	66.03	31.29	0.169	+ 0.100	145	0.170	146	Excited when brought to laboratory
71	M	3.31	61.36	18.54	0.098	+ 0.029	42	0.091	32	In bell jar 3 min. just before killing
90	M	3.45	105.63	30.62	0.133	+ 0.064	93	0.133	93	Slight excitement before killing
92	F	2.10	61.90	29.48	0.149	+ 0.080	116	0.149	116	Excited by confinement in apparatus
108	F	2.19	45.86	20.94	0.122	+ 0.053	77	0.116	68	As 64: also held rigidly 3 min. before killing
110	F	2.20	56.86	25.85	0.086	+ 0.017	25	0.084	22	Held as 108 for 8 min., previously quiet

what has been said, it will be seen that those experiments in the past in which the blood has been drawn from an artery or vein without anesthetics have a very doubtful value, since it is hardly probable that an animal will undergo such an operation and remain in perfect tranquillity. The necessary restraint is of itself sufficient to influence the results as indicated by animals number 108 and 110. Of the disturbing effect of anesthetics more will be said later. There remains then only the possibility of rapidly killing the animal with the least possible excitement and the rapid withdrawal of the sample of blood after death. This was long ago appreciated by Pavy, who killed his animals by pithing with a Bernard needle and then collected the blood from the heart or from the thoracic cavity after severing the large blood vessels. Sudden decapitation and collection of the blood from the severed neck vessels seemed to offer some advantages over Pavy's method, and was used throughout the present work. The interval during which the animal was held before decapitation seldom exceeded three seconds, while about fifteen to twenty seconds more were required for the collection of the blood. As a further precaution against excitement, an attendant, from whom the animals were accustomed to receive food, brought them to the laboratory and assisted throughout the preparation of the animal and the collection of the blood.

That the amount of blood drawn relative to the total amount in the body may affect the concentration of sugar in the sample seems to have been overlooked by previous investigators. In studying this relationship a number of standard experiments were tabulated in the order of the increasing amounts of blood drawn, when this was expressed in grams per kilo of body weight. It was then found that the respective concentrations of sugar were arranged in the reverse order: that is, the more blood drawn per kilo of body weight, the lower is its concentration of sugar. This is wholly independent of the actual amount of blood drawn, as is shown below. The phenomenon is somewhat surprising since we know that hemorrhage under certain conditions causes hyperglycaemia. The relationship of which we are speaking, however, is not to be confused with the so-called "hemorrhage hyperglycaemia" as that term is commonly used.

In the curve shown in figure 1 the data are derived from Table 5. Unfortunately the body weights of the animals used in the earlier experiments were not recorded, hence these experiments are not available for our present use. The amounts of blood drawn

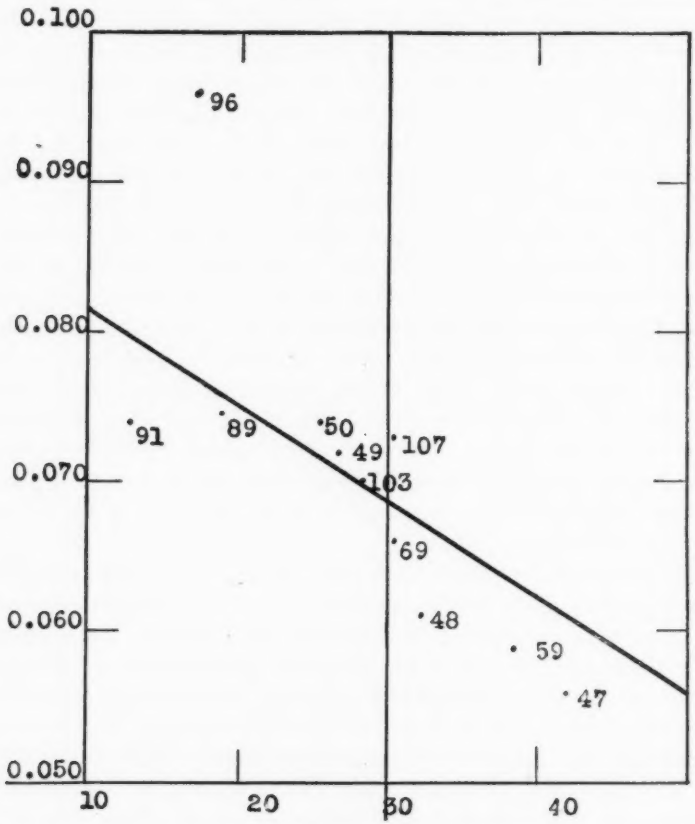

FIGURE 1

per kilo of body weight are plotted on the x-axis. The concentrations of sugar in the blood, expressed in grams per cent, were plotted on the y-axis. Provisionally the line represented by the equation $x/a + y/b = 1$ is taken as representing the relation found. In the equation x and y are variables; x represents the amounts

of blood drawn per kilo of body weight, and y the concentrations of sugar in the blood. a and b are constants whose values have not been exactly determined, but which approximate 133 and 0.084 respectively.

If this relationship between the amount of blood drawn and its concentration of sugar is constant from one animal to another, and if the above formula is its true expression, y is a constant in the equation $y = b\,(x' - x) / a + y'$; where a and b have the values assigned to them above. x' and y' represent respectively the amount of blood drawn per kilo of body weight and its concentration of sugar in any particular experiment. x may have any arbitrary value. Under these circumstances y represents the concentration which would have been found had x grams of blood per kilo of body weight been drawn. This calculation has been made for a number of the experiments, x being taken as equal to 30. The results, when recorded in the tables, are found in the column headed values calculated for 30 grams per kilo body weight. Since there have not been enough estimations made to warrant the assignment of exact values to a and b, the optimum position of the curve was ascertained by trial and the values were found mechanically after having plotted the results on coordinate paper as for figure 1. The results for the standard animals, calculated in the manner just described, are given in Table 5, column 10. A comparison of columns 7 and 10 of this table shows, first, that the mean for the series has not been modified; secondly, that the difference between the highest and the lowest results for the eleven animals compared is greatly reduced. Again the number of individuals which vary from the mean by more than 10 per cent is reduced from 4 to 1, or from 36 per cent to 9 per cent of the whole number of animals compared. Thus it will be seen that in general the calculated values approach still closer to a constant than do those derived directly from the analysis.

In order to test this relationship still further the blood was drawn from four animals in a series of samples in each of which the sugar was determined. The results obtained confirm those obtained by the former method and are given in Table 6. A study of this table reveals that in every case, with the exception of the third sample in experiment 95, the concentration of sugar in any sample

in the series is lower than for any sample in the same series previously drawn. These results do, however, indicate that the curve representing the relation is not a straight line, but that it falls more rapidly at first than it does later.

It was suggested that this relationship is due only to an error in analytical technique and so is of no physiological significance. For instance, it might be that a greater percentage of the sugar is recovered when only small amounts of blood are used. While it is true that such an error might lead to somewhat similar results, the

TABLE 4

THE EFFECT OF A DIET OF BREAD AND MEAT UPON THE CONCENTRATION OF SUGAR IN THE BLOOD OF CATS

No. of Exp.	Sex	Days on diet	Body wt. k.	Blood drawn gm.	Blood per k. body wt. gm.	Gm. % glucose recovered	Var. from standard mean-table 5		Values cal. for 30 gm. blood per k. body wt.	
							Absolute	% of mean	Concentration gm. %	Var. % of standard
45	F	24	2.50	93.29	37.12	0.100	+ 0.031	45	0.105	+ 52
46	M	12	2.94	101.42	34.50	0.070	+ 0.001	1	0.073	+ 6
53	M	24	2.98	83.62	28.06	0.104	+ 0.035	51	0.102	+ 48
54	F	24	2.75	79.55	28.93	0.060	− 0.009	13	0.059	− 14
55	M	24	2.50	72.66	29.06	0.056	− 0.013	19	0.056	− 19
56	M	18	2.94	65.22	22.18	0.080	+ 0.011	16	0.075	+ 9
Mean						0.078	+ 0.009	13	0.078	+ 13

criticism cannot be valid for three reasons. First, it has been found that when the sugar in unequal samples of the same blood was determined, slightly smaller concentrations were found in the smaller samples. This was probably due to some slight negative error which has a tendency to be constant. This error would be multiplied by a larger factor when the absolute amounts recovered were computed to percentages and so lead to the smaller concentrations found in the smaller samples of blood. Thus it will be seen that in so far as this error would have any tendency, it would be to

hide the relationship found rather than to simulate it. Secondly, the effect is the same whether the variation in the amount of blood per kilo of body weight is brought about by drawing different amounts of blood from animals of approximately the same weight or by drawing the same amounts of blood from animals of different weights. Compare experiments 59 with 91, and 69 with 89 in Table 5.

TABLE 5

THE CONCENTRATION OF SUGAR IN CAT'S BLOOD UNDER THE CONDITIONS WHICH WERE SELECTED AS STANDARD

No. of Exp.	Sex	Days on diet	Body wt. k.	Blood drawn gm.	Blood per k. body wt. gm.	Gm. % glucose recovered	Var. from standard mean, table 5		Values cal. for 30 gm. blood per k. body wt.	
							Absolute	% of mean	Concentration gm. %	Var. % of standard
12	M	1	—	97.86	—	0.062	− 0.007	10	—	—
13	M	1	—	94.36	—	0.076	+ 0.007	10	—	—
15	—	1	—	129.73	—	0.063	− 0.006	9	—	—
16	—	1	—	60.94	—	0.063	− 0.006	9	—	—
23	—	5	—	116.33	—	0.070	+ 0.001	1	—	—
47	F	9	2.00	84.07	42.04	0.056	− 0.013	19	0.064	− 7
48	F	14	2.66	86.10	32.37	0.061	− 0.008	12	0.063	− 9
49	F	16	2.46	66.28	26.94	0.072	+ 0.003	4	0.070	+ 1
50	M	16	4.96	125.31	25.26	0.074	+ 0.005	7	0.071	+ 3
59	M	14	3.16	122.95	38.91	0.059	− 0.010	15	0.064	− 7
68	F	18	3.25	55.80	17.17	0.096	+ 0.027	39	0.088	+ 28
69	F	16	2.19	66.77	30.49	0.066	− 0.003	4	0.066	− 4
89	F	6	3.65	65.84	18.04	0.075	+ 0.006	9	0.068	− 1
91	M	25	3.05	39.56	12.97	0.074	+ 0.005	7	0.062	+ 10
103	M	8	2.23	62.61	28.08	0.070	+ 0.001	1	0.069	± 0
107	F	10	2.25	68.35	30.38	0.073	+ 0.004	6	0.073	+ 6
Mean	—	—	2.93	83.93	27.38	0.069	—	—	0.069	—

Thirdly, if the figures given in Table 6 are compared, it will be seen that the progressive decrease in concentration is entirely independent of the absolute amount of blood drawn.

No experiments have yet been made to determine the physiological significance of this decrease in the concentration of sugar when a larger proportion of the total blood is drawn at one time. The most plausible explanation which occurs to me is that it is due to the leaching of the tissue fluids into the blood vessels which occurs during severe hemorrhage. Evidence as to whether such a leaching does occur could be obtained by making simultaneous estimations of the sugar and the hemoglobin in the blood. Variations of the viscosity of the blood might also be used to throw light upon this question. Professor Burton-Opitz has found that blood drawn 25 to 30 minutes after a severe hemorrhage has a lower viscosity than that drawn before the hemorrhage. More than this, he assures me [1] that from his experience he would expect that the last of a large amount of blood drawn at one time would have a noticeably lower viscosity than would the first portions. This harmonizes directly with the theory advanced above.

Increased concentrations caused by the usual methods of withdrawal of blood have without doubt been the reason why the effect of the relative amount of hemorrhage upon the concentration of sugar in the blood has been so long overlooked. Factors are introduced by these methods which cause a greater or less discharge of the stores of glycogen, and so any small diminution in the concentrations of sugar in the blood is hidden. Pavy's method of collection should give the same results as mine, provided all the blood which has flowed from the vessels up to the time of collection is analyzed. However, I have been unable to find all of the necessary data in any of his tables. Schenck reports a very small difference between the first and second of two consecutive samples. In two experiments, the concentration of sugar in the second sample was less than in the first, and in one experiment, greater. Anderson, in two different experiments, finds almost the same concentration of sugar in each of three consecutive samples of blood. The later samples have, however, a slightly greater concentration of sugar than the earlier ones. Pavy [1] reports an increased amount of

[1] Personal communication.

sugar in the later samples of bullock's blood whether the animals were killed by the Jewish or by the pole-axe method. In none of the above were the samples so obtained that the discharge of stored glycogen would have been prevented.

Though probably sex of itself has no influence upon the concentration of sugar in the blood (cf. Bang), it is quite possible that the greater excitability of the male cats which Cannon [1] has found to exist may operate through the mechanism for emotional glycosuria to simulate such an influence. If this were true, it would be especially noticeable in experiments which involve much handling of the animal or its confinement in apparatus. While for animals kept under standard conditions I have found the mean for females slightly higher than that for males, the difference is small. Moreover, the nature of the individual variations, Tables 4 and 5, make one hesitate to attach any significance to this slight difference. It is quite possible that the results would have been otherwise for a series of animals confined in some apparatus, as a respiration chamber, for a period of time. The only evidence that I have on this is drawn from two animals which were confined in a small cage and exposed to a lowered temperature for a period of two hours. A small female cat, number 92, Table 3, resented the treatment and yielded a concentration of 0.149 per cent of sugar, while a large male, number 93, was apparently tranquil throughout the period, and yielded a result even lower than usual, 0.089 per cent. It seems to me that the stress should be laid upon the nature of the particular individual, rather than in blindly choosing animals of either sex. The sex of almost all the animals used is indicated in the tables. The results for some of the longer series are summarized in Table 7.

It is very doubtful whether there is any direct relationship between the body weight of the animal and the concentration of sugar in the blood, provided that the animals used are otherwise comparable. It must be borne in mind that variations in weight may be brought about by an abnormal physical condition, e.g., tuberculosis, and conversely these variations may be used as a means of detecting such abnormal conditions. A study of Tables 4 and 5 or of Table 8, in which the results bearing upon this point are summarized, reveals the fact that if the animals are divided

TABLE 6

THE RELATION BETWEEN THE AMOUNT OF BLOOD DRAWN PER KILO OF BODY WEIGHT AND ITS CONCENTRATION OF SUGAR

	Experiment 89 Body wt. 3.65 k.		Experiment 90 Body wt. 3.45 k.			Experiment 91 Body wt. 3.05 k.		Experiment 95 Body wt. 2.35 k.			
Order drawn	1st	2nd	1st	2nd	3rd	1st	2nd	1st	2nd	3rd	4th
Amount of blood gm.	44.89	20.95	54.2 (?)	29.29	22.04	25.47	14.09	17.55	13.93	10.43	37.79
Concentration of sugar	0.076	0.074	—	0.138	0.133	0.077	0.066	0.058	0.048	0.053	0.041
Remarks			1st sample spilled in tray, collected on weighed cotton and weighed. Not analyzed.								

TABLE 7

RELATION OF SEX TO AMOUNT OF SUGAR IN THE BLOOD

	Normal				Ether						Emotion	
	Meat		Bread and meat		Meat		Bread and meat		Street		Meat	
Sex	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
No. of indiv	6	7	4	2	6	—	4	7	4	4	4	4
Gm. per cent glucose	0.069	0.071	0.077	0.080	0.152	—	0.192	0.166	0.129	0.102	0.120	0.115

into two series, those heavier than the mean and those lighter than the mean, the average concentration of sugar in the blood of the former is slightly higher than in the latter. This difference is, however, slight, and a study of the individual variations indicate that there is no direct relationship between body weight and the concentration of sugar in the blood.

TABLE 8

SHOWING THE INDEPENDENCE OF BODY WEIGHT AND THE CONCENTRATION OF SUGAR IN THE BLOOD OF HEALTHY CATS

	Meat fed Gm. % of sugar		Bread and meat Gm. % of sugar	
	Actual	Calculated to 30 gm.	Actual	Calculated to 30 gm.
Gm. per cent for heaviest animal in series	.074	.071	.105	.102
Gm. per cent for lightest animal in series	.056	.064	.100 .056	.104 .056
Average body wt. for series k.	2.93	2.93	2.77	2.77
Wt. of animal with most sugar	3.25	3.25	2.98	2.50
Wt. of animal with least sugar	2.00	3.05	2.50	2.50
Gm. per cent sugar for those heavier than mean	.076	.071	.085	.083
Gm. per cent sugar for those lighter than mean	.066	.068	.072	.073

IV. — THE CONTENT OF SUGAR IN THE BLOOD OF STANDARD ANIMALS

Before an interpretation of experimental work may be legitimately attempted, a standard must be fixed as a basis for comparison. It must, however, be clearly kept in mind that such a standard is no more normal than many other values which might be obtained. One of the most striking characteristics of almost all of the published tables showing the concentration of sugar in the blood is not the constancy which we have been led to expect, but a variation within rather wide limits.

One of our criteria of life is the ability of the organism to respond to changes in the environment. That is, in any environment, the organism tends to reach a condition of equilibrium and is successful in life in so far as it is successful in maintaining itself in equilibrium with its constantly changing environment. This has long been recognized for external and physical conditions, so that one would hardly say, for example, that an animal was more normal standing than walking, or asleep than awake. But internal, including chemical, readjustments must occur which are just as normal as are the more obvious physical responses. Mathews has recently spoken of the general bearing of this class of adjustments. Cannon [2] has selected the concentration of sugar in the blood of animals undergoing emotional disturbance as a type of such readjustment. He holds, and with reason, that there may be as much "purpose" in this reflex as there is in the accompanying muscular response. Indeed, the increased amount of mobilized sugar may be necessary to make the more obvious muscular response possible.

It is then hopeless to think of finding any one value which will be closely approximated by all normal animals. However this may be, the more nearly we subject the animals to a standard set of conditions before the sample of blood is obtained, the more nearly we may expect to approach a constant value. With organisms so complex as are the mammals, absolute constancy of the preliminary conditions is manifestly impracticable, and so we can hardly expect an absolutely constant value for the concentration of sugar in the blood. Again, after having established a standard value for the concentration of sugar under some one set of conditions, modification of any one or more of the factors might be expected to give a new, but none the less normal, value. Thus the addition of bread to the diet might well give a different value than meat alone (compare Tables 4 and 5).

The method of preliminary treatment which in my hands has given the most constant results, together with some of the factors which may bring about variations, has already been described. The results are given in Table 5 (compare also the values given in Table 1). Results in the other tables are to be compared with those in Table 5 as a standard, since the experiments have been

made upon animals which might otherwise have been presumed to have given similar results.

These considerations, together with the relation between the condition of the sugar in the blood and the method of analysis, make it obvious that at present only relative values for the concentration of sugar in the blood are to be expected. In order that the results of a research may be comparable, a set of preliminary conditions, which have been shown to give an approximately constant concentration of sugar, must be selected as a standard. The exact nature of these conditions will, presumably, be determined, to some extent at least, by the nature of the particular research in hand. Before the results of different researches may be properly compared, they must be reduced to similar terms. This may be done by a factor of comparison similar to the one described on page 277, but which includes the preliminary conditions as well as the method of analysis.

There seem to have been but comparatively few determinations of the sugar in cat's blood. Boehm and Hoffmann made 26 observations on blood drawn from the carotid without anesthesia. Their results are, as one would expect, high, varying between 0.11 per cent and 0.31 per cent. They may well be considered examples of emotional hyperglycaemia and should be compared with my results given in Table 3, rather than with the standard results in Table 5. Rona and Takahashi report analyses of the blood from four cats. They too drew the blood from the carotid, but under light narcosis. The concentrations which they found are quite comparable in magnitude with those of Boehm and Hoffmann, varying between 0.154 per cent and 0.355 per cent. The high concentration here is, however, probably due to the anesthetic and should be compared with results shown in Tables 10–12 rather than with my standard results. Pavy [2] gives the results of six analyses of blood taken from the heart after pithing. The values vary between 0.068 per cent and 0.1026 per cent, with a mean of 0.088 per cent. Since the type of diet and general preliminary treatment are not given, one cannot tell to what extent his results are comparable with mine, or whether they should be compared with my standard or with the results for cats fed on bread and meat which are given in Table 4. The above results are summarized in Table 9.

TABLE 9

TABLE GIVING SUMMARY OF CONCENTRATIONS OF SUGAR IN CAT'S BLOOD FOUND BY DIFFERENT OBSERVERS

Observer	Manner of collection	No. of observations	Mean concentration in %	Highest concentration	Lowest concentration	Observations which vary from average by more than 10%	
						Absolute no.	% of whole no.
Boehm and Hoffmann	From carotid no anesthesia	26	0.15	0.31	0.11	21 (?)	81
Rona and Takahashi	From carotid light narcosis	4	0.282	0.355	0.154	3	75
Pavy	From heart after pithing	6	0.088	0.103	0.068	5	83
Scott	From neck vessels after decapitation	22	0.069	0.096	0.056	4	18

V. — THE RELATION OF A FEW OF THE ORDINARY EXPERIMENTAL PROCEDURES TO THE CONCENTRATION OF SUGAR IN THE BLOOD

From what has been said it will be seen that any experiment which involves the estimation of the sugar in the blood would be valueless if the animal is subjected to pain or other form of excitement during the course of the experiment or within a few hours previous to it. It will also be noted that this is quite apart from any humanitarian considerations. Any work therefore which would otherwise involve pain must be accompanied by an anesthetic. This at once brings up the question of the effect of the anesthetic itself.

That ether, occasionally at least, causes glycosuria in patients undergoing operations has been known almost from the beginning of its use as an anesthetic. Harley and Tiegel very early demonstrated glycosuria in animals to which ether had been given. Hawk maintains that it always occurs in dogs when ether is used as an anesthetic, and his statements have been confirmed by Seelig.

Underhill publishes figures showing an increase in the concentration of sugar in the blood of two dogs which had received ether.

The fact that the administration of ether is accompanied by hyperglycaemia does not of itself preclude its use in experiments of this character. If a set of conditions — of which ether is one — can be found that meets the requirements of a standard, it would seem that the use of ether would be legitimate. In the use of ether, difficulty is at once encountered in getting the animal under the influence of the drug without introducing other disturbing factors. In Table 3 it was shown that the rigid holding of the animal necessary in the use of the cone for this purpose is productive of a significant disturbance in the concentration of sugar in the blood. Likewise, in the single case tried, a similar result was obtained when the animal was confined under a bell jar. This particular animal, however, resented the confinement. It was found that by careful selection of individuals those could be found which so far as one could tell were not disturbed by the brief restraint necessary. The bell jar has the disadvantage of offering greater danger of partial asphyxiation than does the cone, and it has been abundantly shown that asphyxia of itself is sufficient to cause hyperglycaemia (cf. Bang). It was thought that with proper precautions any danger of asphyxia could be avoided and that aside from this there were fewer objections to the use of the bell jar. Consequently in all of my experiments, where ether or chloroform was given, the animal was put in a bell jar for the initial stages. The animals were removed from the jar as soon as muscular relaxation had occurred. When the anesthetic was to be given for a longer time, this was done by means of a cone. Asphyxia was avoided either by very rapid anesthetization in a jar of fairly large volume or by the admission of air below the jar when slower anesthetization was desired.

The results for animals prepared in the standard manner are shown in Table 10. It is evident that there is no approximation to a constant. And in addition to this the animals to which ether was given for 30 minutes have a distinctly higher concentration of sugar in their blood than those to which it was administered for only three minutes or less. This indicates a cumulative effect of the ether, which would still further confuse the results of the experiment.

TABLE 10

THE AMOUNT OF SUGAR IN THE BLOOD OF CATS PREPARED IN STANDARD MANNER + ETHER

No. of Exp.	Sex	No. of days on diet	Body wt. k.	Time in min. from beginning of etherization		Amt. of blood gm.	Concentration glucose gm. %	% var. from		Values calculated. for 30 gm. blood per k.		
				To musc. relax.	To death			Mean this series	Mean stand. series	Concentration	% of var. from mean of	
											This series	Stand. series
66	M	16	3.45	.92	1.00	79.38	0.113	+ 3	+ 64	0.108	+ 1	+ 57
73 [1]	M	12	3.29	2.67	3.00	97.20	0.132	+ 20	+ 91	0.132	+ 23	+ 91
74	M	12	2.50	2.17	2.17 +	59.43	0.086	- 22	+ 25	0.082	- 23	+ 19
Mean	—	—	—	—	—	—	0.110	—	+ 59	0.107	—	+ 55
78 [2]	M	14	3.91	1.50	30	89.45	0.133	- 32	+ 93	0.128	- 34	+ 86
81	M	14	2.10	3.67	30	71.31	0.302	+ 54	+ 338	0.305	+ 56	+ 342
86	M	9	2.80	2.75	30	84.43	0.153	- 22	+ 124	0.153	- 22	+ 124
Mean	—	—	—	—	—	—	0.196	—	+ 184	0.195	—	+ 183

[1] Slight struggling when brought to laboratory.
[2] Shivering last ten minutes.

Seelig reports that ether gives much less trouble in this way with dogs which have been fed on bread for some time than with those which have been on a meat diet. Macleod also has sought to avoid the disturbing effect of ether in the same way. The results which I have obtained with cats fed on the bread and meat diet described on page 281 are shown in Table 11. A comparison of these results with those given in the preceding table shows that while there is still so much variation that they would be unsatisfactory as a basis for experimental results, they are more uniform than those obtained with the meat diet. Also the cumulative effect of the ether is not so great.

A definite relation between the ease with which the equilibrium of the mobile carbohydrates of the body is disturbed and the type of diet given the animal would be of considerable theoretical interest. Such a difference must imply a difference either in the chemical form of the carbohydrate or in the tissues in which it is stored. This theoretical interest, together with the opportunity which might be offered the experimentalist of reducing the variations to a minimum, would warrant sufficient work to establish either the existence or non-existence of such a relation. This is especially true, since Seelig's results agree with those given above in indicating the hopeful outcome of such a research.

The results given in Table 12 were obtained from animals which were to be used by a class of medical students. These animals were killed by decapitation as usual, but without special preparation. The first eight were anesthetized by ether in a bell jar in the usual manner by the students. As soon as muscular relaxation had occurred, they were removed from the jar and decapitated at once. The last five were used for demonstration purposes and had been under ether for periods varying from an hour to three or four hours, during which time the operation indicated had been done. Since these animals were primarily used for other purposes, I was unable to record the full data. The results are, however, given in the hope that they will prove of some value, indicative as they are of the results which may be expected under ordinary laboratory conditions. Chloroform does not seem to offer any advantages over ether (Harley) and has the disadvantage of a greater toxicity. A few experiments of my own, likewise, give no indication of any

TABLE 11

THE CONCENTRATION OF SUGAR IN THE BLOOD OF CATS FED ON BREAD AND MEAT AND WHICH HAVE BEEN GIVEN ETHER

No. of Exp.	Sex	No. of days on diet	Body wt. k.	Time in min. from beginning of etherization		Amt. of blood gm.	Concentration glucose gm. %	% Var. from		Values calculated for 30 gm. blood per k.		
				To musc. relax.	To death			Mean this series	Mean stand. series 0.078	Concentration	% of var. from mean	
											This series	Stand. series 0.078
63	F	11	2.49	1.00	1 +	79.99	0.172	+ 15	+ 120	0.173	+ 16	+ 122
65	F	12	2.45	1.17	1.17 +	63.42	0.135	− 9	+ 73	0.132	− 11	+ 69
67	M	17	2.12	1.67	2.67	53.30	0.170	+ 14	+ 118	0.167	+ 12	+ 114
70	F	19	2.17	3.50	3.50 +	58.82	0.166	+ 11	+ 113	0.164	+ 10	+ 110
72	F	20	1.84	3.00	4.00	52.34	0.142	− 5	+ 82	0.141	− 5	+ 81
79	F	23	2.26	2.17	2.17 +	80.55	0.111	− 25	+ 42	0.115	− 23	+ 47
Mean	—	—	—	—	—	—	0.149	—	+ 91	0.149	—	+ 91
75	M	8	2.02	3.50	24	70.93	0.213	+ 3	+ 173	0.216	+ 6	+ 177
80	M	8	3.35	2.50	30	61.22	0.215	+ 4	+ 175	0.208	+ 2	+ 167
82	F	7	2.80	3.83	67	65.30	0.155	− 25	+ 99	0.150	− 26	+ 92
83	M	7	2.25	1.50	29	57.06	0.171	− 17	+ 119	0.163	− 18	+ 115
94	F	29	2.65	4.00	34	80.48	0.280	+ 35	+ 259	0.280	+ 37	+ 259
Mean	—	—	—	—	—	—	0.207	—	+ 165	0.204	—	+ 161

TABLE 12

EFFECT OF ETHER ON CONCENTRATION OF SUGAR IN BLOOD OF CATS WHICH HAVE RECEIVED NO ESPECIAL PREPARATION

No. of Exp.	Sex	Body wt. k.	Amt. of blood gm.	Concentration sugar gm. %	Per cent of variation from mean of			Remarks
					This series	Stand. series	Bread and meat series	
37	M	—	80.05	0.106	− 8	+ 54	+ 38	
38	F	—	75.25	0.104	− 10	+ 51	+ 33	
39	M	—	97.25	0.151	+ 31	+ 119	+ 94	
40	F	—	52.60	0.094	− 18	+ 36	+ 21	
41	M	—	80.60	0.123	+ 7	+ 78	+ 58	
42	M	—	63.25	0.133	+ 16	+ 93	+ 71	
43	F	—	49.45	0.126	+ 10	+ 83	+ 62	
44	F	—	62.05	0.084	− 27	+ 22	+ 8	
Mean	—	—	—	0.115	—	+ 67	+ 47	
25[1]	—	—	91.25	0.129	− 40	+ 87	+ 65	Pleural puncture, treacheotomy
57[1]	M	3.00	80.89	0.134	− 38	+ 94	+ 72	Decortication
62[1]	M	3.25	55.66	0.239	+ 11	+ 246	+ 206	Decortication less ether than no. 57
84[1]	M	2.80	79.92	0.298	+ 39	+ 332	+ 282	
85[1]	M	2.75	72.80	0.274	+ 27	+ 300	+ 251	Respiration stopped under ether
Mean	—	—	—	0.215	—	+ 212	+ 176	

[1] These animals were used for class demonstration and were under ether for at least one hour and in addition were subjected to the operations indicated.

advantage to be derived from its use, since the results are essentially similar to those obtained by the use of ether (Table 13).

Because of its stimulating action on the cat, no one would think of making use of morphine in drawing blood from this particular animal. It is, however, of interest to note that Luzzatto finds glycosuria following the use of morphine in rabbits. This finding is confirmed by Araki, who also reports similar results for

TABLE 13

THE RELATION BETWEEN CHLOROFORM AND THE CONCENTRATION OF SUGAR IN THE BLOOD OF ANIMALS PREPARED IN THE STANDARD MANNER

No. of Exp.	Sex	Body wt. k.	Time in min. from application of chloroform to		Amt. of blood drawn gm.	Concentration sugar gm. %	% var. from mean		Remarks
			Musc. relaxation	Death			This series	Stand. series	
87	M	3.79	5.0	5.17	78.05	0.105	− 9	+ 52	Unusually quiet before and during anesthetization
88	M	3.50	2.0	2.67	67.94	0.098	− 15	+ 42	
97	F	2.75	1.5	1.57	62.96	0.142	+ 23	+ 105	
Mean	—	—	—	—	—	0.115	—	+ 67	

dogs, though he found no sugar in the urine of frogs after morphine. On the other hand, Hirsch and Reinbach think that morphine is without effect on the concentration of sugar in the blood of rabbits. Jacobsen found an undoubted increase in the amount of sugar in the blood of rabbits to which sufficient chloral had been given to produce narcosis.

Some investigators have collected blood from one of the large vessels under the local anesthesia produced by cocaine (Fisher and Wishart). For some types of experiment, such a method is particularly desirable, provided the equilibrium of the mobile sugar is not disturbed by the drug in such a manner that the proper allowances cannot be made. Araki found lactic acid in the urine of frogs and of rabbits after the injection of cocaine. One of the four rabbits injected also secreted sugar with the urine. In the present work four cats were injected beneath the skin of the back with large doses of cocain hydrochloride dissolved in N/8 sodium chloride solution. These animals were all killed in the early stages of the apparent reaction to the drug. (See Table 14 for details.) With one exception each of the concentrations of sugar found was well below the standard concentration. The mean concentration for the series is 86 per cent of the standard mean. Any attempt to explain this finding would be premature, since a longer series

TABLE 14

THE EFFECT OF COCAINE UPON THE CONCENTRATION OF SUGAR IN THE BLOOD OF CATS PREPARED IN THE STANDARD MANNER

No. of Exp.	Sex	Days on diet	Body wt. k.	Amt. cocaine injected gm.	Time between injection and death in min.	Amt. of blood drawn gm.	Concentration sugar in blood gm. %	% Var. from mean of		Remarks
								This series	Stand. series	
98	F	2	2.58	0.07	8	68.79	0.063	+ 7	− 9	Killed in 1st stages of stimulation by drug
100 [1]	M	3	2.85	0.05	10	87.40	0.055	− 7	− 20	No other excitement
101	F	6	2.37	0.07	6	57.38	0.048	− 19	− 30	As No. 98
102	M	8	2.68	0.07	6	77.85	0.070	+ 19	+ 1	Killed after symptoms of the drug were marked
Mean	—	—	—	—	—	—	0.059	—	− 14	

[1] Only slight symptoms of drug at time of death.

might yield results which would essentially modify the situation. Also too little is known at present of the other factors of metabolism during intoxication by cocaine to warrant such an attempt.

In following the progress of an experiment it is frequently desirable to determine the changes in the concentration of the sugar in the blood at frequent intervals. Unfortunately there is, however, a very serious objection to this procedure. Claude Bernard found that the concentration of sugar is increased by a previous hemorrhage, and his finding has been repeatedly confirmed. Recently fairly exhaustive studies have been made by several authors. Among others Anderson, Jacobsen, Rose and Schenck have studied this effect in rabbits. Anderson found an increased concentration of sugar five minutes after the hemorrhage, but did not determine whether it was present after a still shorter interval. The consensus of opinion is that the concentration reaches its maximum about thirty minutes after the hemorrhage and that it remains high from three to four hours.

Undoubtedly emotional disturbances have frequently contributed a large share to the so-called hemorrhage hyperglycaemia. However this may be, there is no doubt that quite apart from any disturbance due to emotion or to anesthetics, hemorrhage does introduce a modification for which proper controls must be made. Some authors have sought to avoid the introduction of the factor of hemorrhage by the use of a quantity of blood so small that it might be considered as negligible, but this so greatly increases the probable error from analytical technique that the method has a questionable value, at least for most methods of analysis. Furthermore the objection to repeated handling of the animal and the consequent excitement are not met by the change in analytical method, and demand exceptional skill on the part of the experimenter. The literature covering this subject is so ample and, taken as a whole, so conclusive that it was not thought necessary to add to it.

Changes in the concentration of the sugar in the blood may be used as a measure of the effect of a substance which has been injected into the animal. In such experiments it is usually presumed that the effect of the injection aside from the drug is nil. My own experiments are too few in number to allow of general conclusions.

But in harmony with the rest of my work they indicate the necessity of complete control of all factors in the experiment. Long ago Bock and Hoffmann showed that large amounts of salt solution caused glycosuria when injected intravenously. But while drugs are frequently dissolved in a solution of sodium chloride for injection, the effect of the salt solution is essentially different from that obtained by Bock and Hoffmann, since usually very much smaller amounts are injected. In my own experiments with cocaine certainly no factor was introduced which increased the concentration of sugar enough to conceal the results due to the cocaine alone, with the possible exception of one animal. (See Table 14.) Especial care was taken in making these injections of cocaine to avoid exciting the animal. The same care was exercised in an animal which was injected with 5 cc. of M/8 sodium chloride. This cat was killed five hours later, and the blood yielded a concentration of sugar of 0.0697 per cent, a result almost exactly the same as the standard. Another animal injected through an opening in a small box in which it was confined with the same amount of sodium chloride solution became much excited. This animal, after the lapse of a similar interval, yielded a concentration of 0.098 per cent — a much higher result than the standard.

Again, many experiments of this nature involve the confinement of the animal within some form of apparatus. The exact results obtained in animal calorimetry are ample evidence of the availability of this type of research. On the other hand, great care is necessary to avoid exciting the animal. This is illustrated in the two animals exposed to cold as described on page 291. While they were exposed to similar external conditions, one became very restless and yielded a concentration of 0.149 per cent of sugar; the other remained exceptionally quiet and yielded a concentration of only 0.049 per cent. The nature of this experiment, together with the results, would suggest the possibility that the unpleasant conditions involved constitute at least one of the factors leading to the mobilization of the sugar in Lusk's method of ridding the body of glycogen by shivering.

In another experiment four cats were confined in a respiratory chamber and subjected to a temperature of about 32° C. and a relative humidity of about 88 per cent. The results are shown in

Table 15. Animals were selected for these experiments which might be expected to remain quiet throughout the experiment. No. 112 was the only one which proved disappointing in this regard. The results show an exceptionally low average for the concentration of sugar, and this individual is the only one of the four which reached the level of the standard cats.

TABLE 15

THE EFFECT OF THE CONFINEMENT OF CATS IN A WARM, MOIST CHAMBER UPON THE CONCENTRATION OF SUGAR IN THE BLOOD

No. of Exp.	Sex	Body wt. k.	Amt. of blood drawn gm.	Hrs. in chamber	Temp. mean C.	Rel. humidity mean	Concentration sugar in blood gm. %	% of var. from stand. mean	Cal. for 30 gm. blood per k. body wt.	
									Concentration sugar gm. %	% var. from stand. average
111	F	3.65	83.38	6	30.6	.83	0.053	– 23	0.049	– 29
112[1]	F	1.62	55.57	6	30.7	.89	0.064	– 7	0.067	– 3
113	F	3.78	69.87	6	33.1	.90	0.065	– 6	0.058	– 16
114	F	2.90	65.12	6	32.9	.90	0.059	– 14	0.054	– 22
Mean	—	—	—	—	31.8	.88	0.060	– 13	0.057	– 16

[1] Excited when removed from the chamber.

There seems then to be no reason for attributing changes in the concentration of the sugar in the blood following the injection of small amounts of salt, or the confinement of the animal in apparatus to these conditions of themselves. Excitement induced by these conditions may, however, give rise to high concentrations, even five hours after the time of irritation.

VI. — SUMMARY AND CONCLUSIONS

1. Glycaemia offers a more satisfactory indication of the condition of mobile sugar than does glycosuria; first, because either an increase or a decrease in the amount of sugar may be demonstrated, while normal urine can show only an increase; secondly,

because profound changes in glycaemia may occur in response to conditions which do not produce glycosuria; thirdly, the blood is in much more direct relation to the living cells than is the urine.

2. The concentration of sugar in the blood as estimated by different methods varies. This may be due, in part at least, to the form in which the sugar is present in the blood. It follows that results obtained by different methods of analysis cannot properly be compared until they have been reduced to common terms. Also the possibility is introduced of an apparent variation in concentration of sugar, even when the method of estimation is constant, which is due to a change in the form in which the sugar is present rather than to a change in the actual amount of sugar present.

3. If consistent results are to be expected, the animals must be uniformly healthy, and must be killed without pain or excitement. Sex or weight, apart from correlated conditions, are probably without special influence upon the concentration of sugar in the blood.

4. The normal concentration of sugar may very probably vary with the varying environment of the animal or with changes in its physical state. However, if the environment is uniform and if the animals are killed while in the same physiological condition, constant results should be expected. Practically such an ideal result is not possible, but has been approached with some success.

5. The concentration of sugar in the blood decreases as the amount of blood drawn per kilo of body weight of the animal increases. So far sufficient data have not been obtained to establish the mathematical expression for this relation.

6. When ether or chloroform was administered, the concentration of sugar was increased considerably and varied between rather wide limits, whether the diet consisted of meat alone, or of bread and meat, the latter diet giving somewhat smaller variations than the former. After either diet there was a greater concentration after the drug had been administered for thirty minutes than after it had been administered for three minutes or less.

7. The concentration of sugar in the blood after subcutaneous injection of cocaine is more constant than that found after inhalation of ether or chloroform and is lower than that found in animals similarly treated but to which cocaine has not been given.

8. It may be shown from the literature that hyperglycaemia

follows hemorrhage. From this it follows that caution must be exercised in drawing conclusions from experiments which involve the analysis of successive samples of blood.

9. The excitement which is apt to attend hypodermic injections or confinement in apparatus may lead to high results and consequently to false conclusions. With care, however, such effects may be avoided so that this type of experiment is permissible.

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THE EFFECT OF CALCIUM AND PROTEIN FED PREGNANT SWINE UPON THE SIZE, VIGOR, BONE, COAT AND CONDITION OF THE OFFSPRING[1]

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[*Animal Husbandry and Chemical Sections, Iowa Experiment Station*]

Received for publication April 22, 1914.

TO determine the effect of adding calcium and protein to a corn ration fed the pregnant gilt upon the relative size and vigor of the offspring, a series of experiments has been conducted at this Station. The results obtained during the year 1912–13 will be discussed briefly.

The gilts under observation were divided into three lots of ten each. Lot I received whole corn grain (shelled) only, 1279.13 grams (reduced to 14 per cent moisture basis) per head daily; Lot II whole corn grain (shelled) the same amount as Lot I, plus calcium allowed in the form of chloride and carbonate (equivalent to approximately 2½ grams of calcium daily); and Lot III corn grain (shelled) the same in amount as Lot I plus protein fed as black albumen to the extent of 136.08 grams of the blood product per head daily. This black albumen analyzed 88.24[4] per cent

[1] Written April 1, 1914. Preliminary Report.

[2] Animal Husbandry Section.

[3] Chemical Section.

[4] The black albumen and corn as analyzed contained in a hundred grams. We used the black albumen because it was the best and purest form of commercial protein on the market. We preferred the blood derivative to the wheat gluten because of the much greater likelihood of its containing the complete series of amino acids.

The blood albumen runs higher than corn only in protein and ash. Fortunately the ash constituents of the corn exceed in potassium, magnesium, and phosphorus. The blood ash excels in sodium, chlorine, calcium and sulphur, but the possible influence of the first two, sodium and chlorine, is

protein and contained very little of the mineral elements, being especially low in calcium. All gilts received equal quantity of sodium chloride daily, namely 7.26 grams per head. The daily gains of the three groups were as follows: Lot I, 107.95; Lot II, 154.68; Lot III, 237.23 grams.

The number of pigs farrowed per sow from these three lots was, respectively: Lot I, average 7.88; Lot II, 7.30; and Lot III, 8.22. Here we notice, as in our previous experiments, that the protein added to the corn ration during the breeding season influences favorably the number of young.

The weight of the total litters, as well as that of the individual pigs, shows clearly the influence of calcium and protein respectively upon the developing fetus. The table presented on page 314 gives the number in litter, litter weight, and average weight per pig. The basis is grams.

negligible because we purposely fed a sufficiency of sodium chloride, the same to all lots. The calcium difference is so small as to be almost negligible. The results presented, wherein Lots I and II are contrasted, show plainly that calcium has some influence, and we must make some allowance for the

	Albumen	Corn
Protein	88.24	9.81
Ether extract	1.30	2.64
Ash (total)	3.26	1.42
Nitrogen free extract	none	74.83
Crude fibre	none	2.38
Moisture	7.20	8.92
Calcium	.03	.01
Phosphorus	.19	.61

extremely small but nevertheless constant difference. As regards the sulphur difference we attribute to it considerable possible influence, — but inasmuch as sulphur is to the protein much as "the tail is to the entire hide" we must charge the effects produced to the protein of the blood albumen. Summarizing, therefore, we find that the results secured by supplementing corn with black albumen are theoretically due almost entirely to its protein content.

WEIGHT OF OFFSPRING

Lot no.	No. in litter	Litter weight grams	Average per pig [1] grams
I	7.88	6454.62	821.00
II	7.30	6695.02	916.26
III	8.22	7838.08	952.54

[1] On basis of all pigs farrowed.

The litter of the lightest weight comes from the group receiving "corn alone," whereas the heaviest is to be found where corn was supplemented with protein as in Lot III. Here we note a litter difference of 1383.46 grams in favor of the protein supplemented corn ration. The protein increased the weight of litter practically 29 per cent. The effect of the complex protein in black albumen is much more marked than that of the simple calcium fed as chloride and carbonate. The important deductions are such as to emphasize the necessity of both of these constituents, namely calcium and protein, in feeding corn to bred swine; the addition of either one of these resulting in heavier litters and larger average pigs.

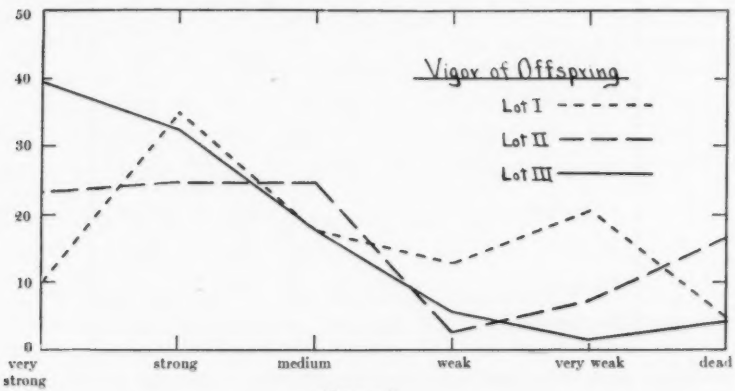


CHART 1

The vigor of the offspring was markedly affected by the ration. We have the following distribution of the pigs in the three lots according to their relative strength:

VIGOR OF OFFSPRING
(On basis of 100 pigs farrowed)

Lot no.	Very strong	Strong	Medium	Weak	Very weak	Dead
I	9.52	34.92	17.46	12.71	20.63	4.76
II	23.29	24.66	24.66	2.74	8.22	16.44
III	39.19	32.43	17.57	5.41	1.35	4.05

Most assuredly the addition of protein affected profoundly the vigor and stamina of the offspring. (See chart I for distribution.) The addition of calcium was not without its effects. The protein, however, seems to be the more important constituent in balancing up the corn when compared to calcium.

The size of bone was likewise affected. When calcium and protein were added to the ration the bones were larger. This was determined by measuring the front and hind shins. The measurements are presented in centimeters:

SIZE OF BONE CIRCUMFERENCE

	Front Shin	Hind Shin
Lot I	4.60	4.36
Lot II	4.88	4.67
Lot III	4.81	4.56

Peculiarly enough, where calcium was added to corn the size of bone was somewhat greater [1] than where the protein was added. Now this may be due in part to the fact that the Lot III farrowed

[1] Cf. Hart, Steenbock, and Fuller, Research Bulletin 30, Wisconsin Experiment Station. "High calcium rations, as compared with low calcium rations, had no effect whatever during a single gestation period on the size or calcium content of the skeleton of the fetus. The skeleton is not increased in any dimension by a wide variation in the amount of calcium fed the mother." According to these investigations the ration considered as a "low calcium" one is a much higher carrier of calcium than the basal ration of corn used in the Iowa experiments.

a greater average number of pigs per litter which would have a tendency, other things being equal, to decrease their relative size.

One is not surprised particularly to find that both calcium and protein had considerable effect upon the size, vigor, and bone of the offspring, but the fact that the coat is likewise markedly affected is somewhat surprising. To determine the influence of the addition of the constituents above mentioned upon the quantity of coat produced in the offspring, observation being made at farrowing time, the relative coat covering upon all of the new-born pigs was carefully recorded. The table on "Coat Quantity of Offspring"

COAT QUANTITY OF OFFSPRING
(On basis of 100 pigs farrowed)

Lot no.	Very heavy	Heavy	Medium	Light	Very light	Absent
I	3.23	29.03	41.94	24.19	1.61	none
II	8.33	34.72	40.28	9.72	6.94	none
III	21.62	40.54	32.43	5.41	none	none

gives the number of pigs out of every hundred born showing the Very Heavy, Heavy, Medium, Light, Very Light and Absent coats. A chart showing the coat quantity distribution is here given.

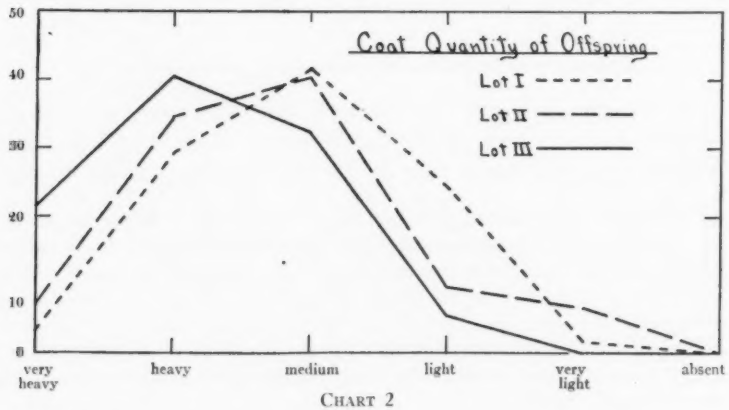


CHART 2

The calcium addition was somewhat effectual in that the coats produced from this lot were a bit heavier. The difference between Lots I and II is, however, very slight. Marked effects are shown from the protein addition where the number having very heavy coats was increased from 3.23 to 21.62 or practically seven times as many possessing the Very Heaviest, Densest coats where protein is allowed as compared to where it was not. Dropping down to the next coat quantity, namely Heavy, we find 29.03 in the check lot as compared to 40.54 where the protein was added, or more than 40 per cent difference. The Very Light coated pigs are conspicuous in Lot III for their absence, thus further demonstrating the effects of protein additions in increasing the amount of hair covering.

That the coat of swine should vary according to the feed given is common experience. Just one month after these young gilts were placed on the experimental rations a marked contrast in the quantity and color of the coats was evident. The coats of hair in the order of their length and density are from Lots III, II, I, with II and I fairly close and III easily first. In color we have the same order, III, II, I, with III much the darkest. It is significant that the coat quantity and color should be affected by the ration, — it is still more suggestive that the coats of the new-born should correspond somewhat with those of the dams from which they were farrowed.

What is the explanation of this difference? We know that keratin, a simple protein of albuminoid nature, is the chief constituent of hair. We find keratin in the epidermis, wool, nails, hoofs, horns, feathers, and so on. Keratin is peculiar in that it has a high sulphur content, the sulphur being present largely in the form of the complex amino acid cystine.

The keratin of human hair runs as high in cystine as 13 to 14½ per cent.[1] No other protein runs so high in cystine as the keratin of human hair. Swine hair or bristles contain about 7.2 per cent cystine. Most assuredly hair cannot be built unless the constituents of cystine are present in the feed, hence it is reasonable to suppose that if said sulphur compound, namely the amino acid cystine, is absent from the feed, the development of hair may be

[1] BUCHTALA: Z. Physiol. Chem., Volume 52, page 474, 1907.

retarded. In corn we find approximately .171 [1] parts of sulphur in 100 parts of dry matter, whereas in black albumen we have .820 [1] parts or almost five times as much, furthermore it has been shown that of the sulphur present in zein, the protein that comprises 58 per cent of the proteins of corn, only 35 per cent [2] is present as cystine. On the other hand a large proportion of the sulphur found in black albumen is supposedly present as cystine, hence it is not unreasonable to assume that the addition of black albumen furnishes the cystine, the basal constituent of hair growth.

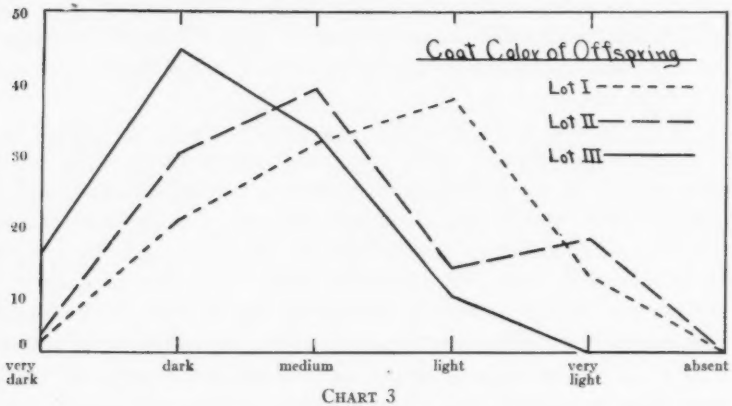


CHART 3

The coat color of the offspring differs, depending upon the dietetic treatment accorded the pregnant dam. The relative effects of the supplements upon the color is illustrated quite clearly in the table on the opposite page showing the number of pigs out of a hundred farrowed classified as Very Dark, Dark, Medium, Very Light, and Absent coat colors:

Again we see the effects of the added black albumen in that it increases the general coat color of the offspring. The chart showing color distribution plainly demonstrates the differences. The hogs which we used were Duroc Jerseys, having red coats. The coats designated as "Very Light" refer to those of little color as compared to the "Very Dark" coats which were of a bright cherry red.

[1] FORBES: Bulletin No. 255, page 225, January, 1913, Ohio Experiment Station.

[2] BUCHTALA: Z. Physiol. Chem., Volume 52, page 474, 1907.

COAT COLOR OF OFFSPRING
(On basis of 100 pigs farrowed)

Lot no.	Very dark	Dark	Medium	Light	Very light	Absent
I	1.61	19.35	30.65	37.10	11.29	none
II	2.78	29.17	38.89	12.50	16.67	none
III	14.86	44.59	32.43	8.11	none	none

The calcium did not seem to affect the coat very much, although it shows a minor influence. The black albumen with its high protein, and possibly its specific cystine content, seems to be the causative agent in the production of highly colored coats. It is to be understood that the coat color markings are affected by the amount of coat present, depending upon whether the hairs are densely studded on the surface of the body as well as the length of the hair, and furthermore on the inherent color of the hair itself. As far as superficial observation goes, without entering into the details of microscopic technical examinations, we would give it as our judgment that the coats were not only denser and longer but that the hairs themselves seemed to show a greater amount of pigment when corn was supplemented with the black albumen protein as compared to corn fed alone.

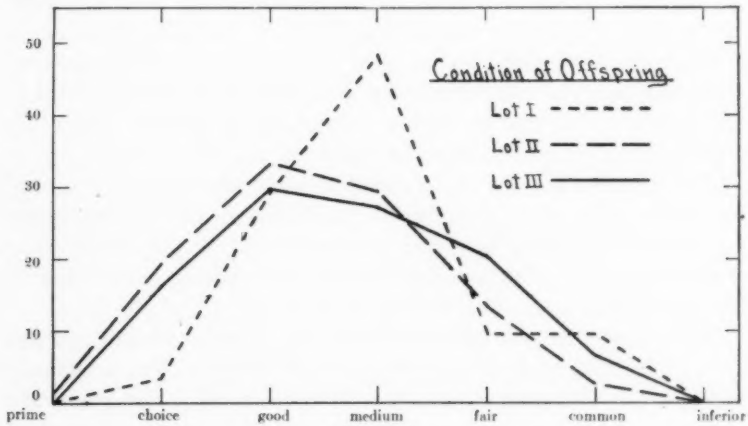


CHART 4

The condition or degree of fatness of the new-born pigs is somewhat dependent upon the feed allowed the dam during the period of gestation. To demonstrate the effect of specific supplements to corn upon the relative condition of the offspring we append herewith table showing the degree of fatness of the various new-born pigs farrowed in the three lots:

CONDITION OF OFFSPRING
(On basis of 100 pigs farrowed)

Lot no.	Prime	Choice	Good	Medium	Fair	Common	Inferior
I	none	3.23	29.03	48.39	9.68	9.68	none
II	1.39	19.44	33.33	29.17	13.89	2.78	none
III	none	16.22	29.73	27.03	20.27	6.76	none

The condition or fatness of the new-born pigs was determined by sight and touch observations. Each pig was handled so that a fairly accurate estimate could be made of the fatty covering, special emphasis being placed upon the superficial layers over the ribs and back. Both the calcium and protein supplements to corn resulted in fatter offspring. The protein in this case had less effect than the calcium. Our estimates of the condition of the dams producing these pigs placed the lots in order of fattest first, thus: III, II, I, whereas the condition of the pigs farrowed of said dams, placing the fattest first, is II, III, I. In other words the condition of the resulting offspring does not compare as closely with that of the mother as does the coat character. There are obvious fundamental reasons for this.

To recapitulate so as to put the foregoing vigor, coat, and condition story on a comparative and more easily interpretable basis there is summarized the relative effects of the specific feed constituents in a grouped combination table chart. (See Chart 5 on opposite page.)

The perpendicular columns denote the average on the assumption of the highest marking being perfect, or 100. The average is computed by placing a value on the various markings given the individual pigs, — thus for vigor the Very Strong pig is credited

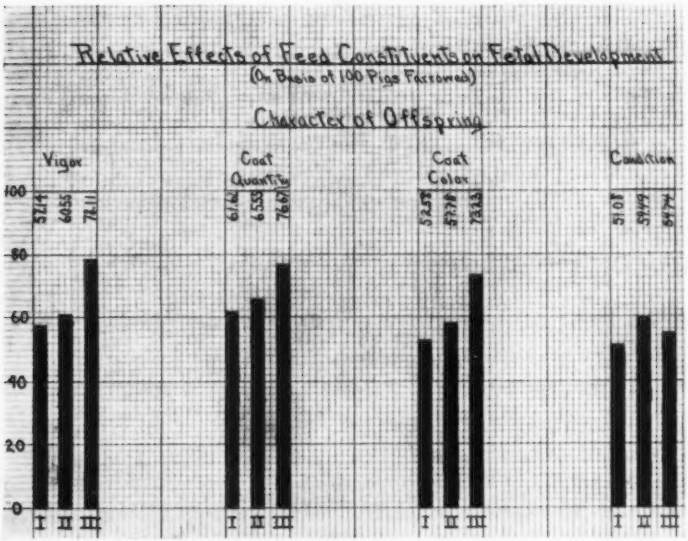


CHART 5

with 100, Strong 80, Medium 60, Weak 40, Very Weak 20, and Dead 0. The Dead with 0 and the Very Strong with the 100 credit makes the range from Absent vigor, the lowest, to Very Strong vigor, the highest marking. The total vigor credits are added and the average taken with results in Lots I, II and III, respectively, of 57.14, 60.55, and 78.11. These values may be regarded as percentages of the maximum vigor marking, and so on.

The same general scheme was followed out in determining the average "Coat Quantity," "Coat Color," and "Condition." The gradations considered are identical with those on the tables and charts previously presented.

Withal, this method gives us a tangible, definite, interpretable average valuation quite in accord with the facts.

Uniformly the supplemental calcium and protein, respectively, produced improvement in specific characters of the offspring. Manifestly the influence of the complex nitrogenous organic constituent protein is more marked than that of the more simple inorganic calcium (chloride and carbonate).

The relative influence of calcium and protein is more clearly appreciated on examination of the following table: —

COMPARATIVE INFLUENCES OF CALCIUM AND PROTEIN FED THE PREGNANT DAM ON DEVELOPING FETUS

Character of offspring	Percentage increase over corn alone attributable to	
	Calcium	Protein
Vigor	5.97	35.00
Coat quantity	6.38	24.42
Coat color	9.89	38.04
Condition	16.46	7.17

Perhaps the direct comparison of protein to calcium effectiveness would make the relation of these two constituents clearer.

The increase of the Protein-Corn-Lot III over the Calcium-Corn-Lot II shows for

Vigor	29.00	per cent
Coat quantity	16.96	" "
Coat color	26.74	" "
Condition	7.90	" "

The protein is more effective than the calcium in the promotion of vigor, production of coat quantity and color, but less so in augmenting the condition.

Evidently the protein is the more efficacious when it comes to the production of those qualities which make for stamina and hardiness. The vigor and coat quantity are relatively more important in lessening the mortality of the suckling than is the degree of fatness. If the new-born be strong, healthy, and well-coated, even though he come into existence under adverse conditions he is much better adapted to live in the environment he finds than if he lacks vigor and coat but possesses a high degree of fatness. The strong, warmly coated pig will soon fatten on his mother's milk, hence the condition comes quickly. Not so however with

the strength and coat; lost vitality and scant hair covering are replaced with comparative slowness.

It is vital to early development that the new-born pigs be vigorous, otherwise they will be compelled to suckle the teats discarded by the more active individuals in the litter. "That pig which suckles the hind teat" is at a disadvantage, but this is the consequence, usually, of being farrowed as the weakly member of the litter.

The protein in corn has been demonstrated to be deficient to a considerable extent in some of the essential amino acids. This is especially true of the zein which comprises practically 58 per cent of the corn proteins, since zein does not contain in its amino acid make-up tryptophane, lysine, and glycine. Fortunately for corn the glutelin which furnishes most of the remaining protein is quite complete in its amino acid constitution. However, the marked preponderance of zein in corn lessens greatly the general efficiency of the protein in toto. The tryptophane [1] is probably the limiting amino acid, hence it is reasonable to assume that the addition to the corn ration of a protein rich in tryptophane would show marked results. We are led to believe from the work already done on the amino acid content of blood and its derivatives that the blood albumen used as the source of protein in our work carries the deficient tryptophane. Perhaps the possible deficiency of cystine in corn as heretofore noted may be a factor, the absence of which contributes to the general inefficiency of the corn proteins. The balancing therefore of the protein present in corn by making it more complete, as well as an increase in the entire amount fed, should be a double reason for the greater efficiency observed.

We had some difficulty in the administration of our calcium. We first started out with calcium chloride but found that where it accompanied protein, given in the form of black albumen, difficulty was experienced in that the mixture seemed to have antagonistic relations. We have supposed that this may possibly be due to acidosis caused by the liberation of the chlorine portion of the calcium chloride molecule, thus freeing hydrochloric acid. Along with a high protein ration the demand for calcium would necessarily

[1] OSBORNE: "The Nutritive Value of the Proteins of Maize" — Science N. S. 1913, xxxvii, page 185.

be greater than where no extra protein was fed, hence we should expect a greater demand for calcium under these conditions with a correspondingly greater liberation of chlorine which would induce acidosis. This acidosis would theoretically be largely done away with by the feeding of a pure calcium limestone such as calcium carbonate. We found when calcium chloride was replaced with calcium carbonate, feeding same between meals, that the ill effects heretofore noted were largely eliminated. Observation and trial showed however that calcium carbonate should not be mixed with the feeds as allowed but that it should be fed preferably between meals. We are further investigating this problem in order to demonstrate the best way to feed the calcium.

It is reasonable to suppose that calcium will give results when added to the corn ration as corn is especially lacking in this important mineral element which comprises 40 per cent of the dry ash of bone. Calcium furnishes 70 per cent of the basal elements of bone, the remaining 29½ per cent being supplied by phosphorus and ½ per cent by magnesium. In the normal human body there is just about two-thirds as much calcium as nitrogen, that fundamental element of protein concerning which we hear so much and upon which a maximum of emphasis is invariably placed by feeding experts and dieticians. It is not to be gainsaid that the lack of protein is the more conspicuous deficiency in ordinary grain diets, — but nevertheless the calcium deserves among the mineral nutrients considerably more attention than is now accorded.

Much of a conflicting nature has been said by obstetricians, dieticians, and the laity concerning the effect of different food constituents upon the development of the embryo and fetus.

The experience at the Iowa Station, involving over 2000 newborn pigs, shows beyond all reasonable doubt that the addition of meat to the ordinary cereal diet of pregnant swine has very marked influence upon the size and vigor of the new-born.

All work heretofore done at the Iowa Station has plainly indicated that the addition of mineral elements as well as protein to the ration had its marked effects upon the development of the young in utero. We are led to believe that any feed, including water, added to or subtracted from the ration of pregnant swine which will tend to promote or discourage growth, thrift, and vigor

of the dam will within reasonable limits have its effect upon the developing fetus.

Summary

1. Corn maize is markedly deficient in calcium and quite low in protein, the major part of which lacks certain important amino acids.

2. The addition of calcium (allowed as chloride and carbonate) to a fixed basal ration of corn and sodium chloride with pregnant gilts resulted in new-born pigs having greater size, more vigor, bigger bone, increased coat quantity, better coat color, and higher condition.

3. The addition of a high protein feed (Black blood albumen) resulted in the new-born pigs having greater size, more vigor, bigger bone, increased coat quantity, better coat color and higher condition.

4. The influence of the complex organic protein is more marked generally than that of the more simple inorganic calcium.

5. The use of chloride as the source of calcium was not as satisfactory as the carbonate in a high protein ration, presumably because of the undesirable liberation of chlorine causing a possible condition of acidosis.

6. The ration fed the pregnant mother affects in a marked degree the general development of the fetus.

THE INFLUENCE OF ADRENALIN ON RESPIRATION

By L. B. NICE, JOHN L. ROCK and R. O. COURTRIGHT

[From the Laboratory of Physiology in the University of Oklahoma]

Received for publication March 30, 1914

IT has been shown by several investigators that the introduction of small doses of adrenalin into the circulatory system causes a fall in blood pressure.[1] Large doses on the other hand cause a rise in blood pressure.[2] As to the effects of adrenalin on the respiratory system, comparatively little work has been done. Oliver and Schäfer[3] found that extracts of adrenal glands cause a shallowness in the depth of respiration. Langley confirmed this result, stating that the respiratory mechanism responds readily to the first injection, but that succeeding injections bring forth insignificant responses.[4] Similar results were obtained by Badano,[5] and later by Boruttan[6] and Kahn.[7] All of these experimenters used large doses of adrenalin.

The purpose of the present study was to investigate the effects of small (physiological) doses of adrenalin on the respiratory mechanism. In order to ensure that the doses were being given accurately and uniformly, the effects on the blood pressure were recorded, as well as those on respiration.

[1] Moore and Purington: Archiv für die gesammte physiologie, 1900, lxxxi, p. 483; Elliott: Journal of physiology, 1905, xxxii, p. 411; Dale: Journal of physiology, 1905, xxxii, p. 59, 1906, xxxiv, p. 169; Cannon and Nice: This journal, 1912, xxix, p. xxiv; Hoskins and McClure: Archives of internal medicine, 1912, x, p. 353; Elliott: Journal of physiology, 1912, xliv, p. 405.

[2] For a general discussion see Vincent: Internal secretion and the ductless glands, London, 1912.

[3] Oliver and Schäfer: Journal of physiology, 1895, xviii, p. 235.

[4] Langley: Journal of physiology, 1901–02, xxvii, p. 253.

[5] Badano: La Clinica Medica Italiana, 1898, p. 375.

[6] Boruttan: Pflüger's Archiv, 1899, lxxviii.

[7] Kahn: Archiv für physiologie, 1903, p. 530.

Methods

Cats and dogs were used in the experiments. The preparations were made in the following way. An animal was fastened back-downward to an animal holder and given urethane (2 gm. per kilo of body weight) by stomach. As soon as anesthetised, a cannula was inserted into a femoral artery, and a mercury manometer attached for recording the blood pressure. A second cannula was inserted into an external jugular vein deep down in the neck. Through this cannula the adrenalin solution was injected.

The abdominal cavity was opened by a median incision, and an S-shaped hook attached to the diaphragm about midway between the central tendon and the lateral chest wall. From the S-shaped hook a thread was passed over a pulley to a writing lever which recorded the movements of the diaphragm on a revolving drum.

The movements of the chest wall were not recorded, as in a previous series of experiments on cats it had been found by one of us,[1] either to move synchronously with the diaphragm or not to move at all. Besides, comparative and not absolute depths of respiration were desired.

The adrenalin used was that of Parke Davis and Co., and was fresh. The strength of solution injected was chiefly a 1:100,000, made by diluting the 1:1000 stock solution with distilled water just before using. In some of the experiments, however, 1:50,000, 1:25,000 and even 1:1000 solutions were used. The injections were made into an external jugular vein at a uniform rate (0.2 c.c. per second) by means of a small syringe having a graduated barrel.[2]

Results

The Relation of the Depth of Respiration to the Fall in Blood Pressure

When 0.3 c.c. or slightly less, of a 1:100,000 adrenalin solution was injected at a uniform rate (0.2 c.c. per second), into a cat,

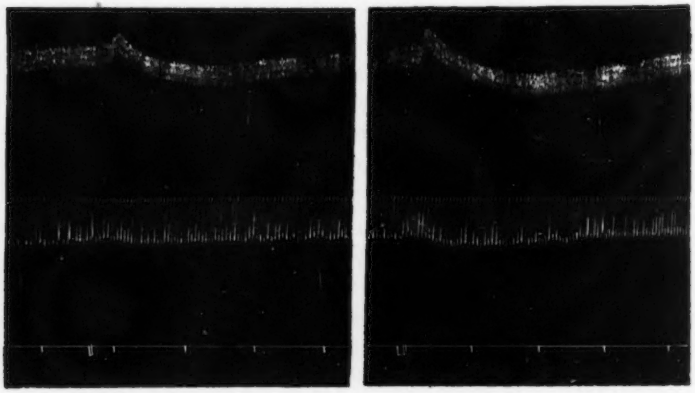
[1] Nice: This journal, 1914, xxxiii, p. 204.

[2] See Cannon and Lyman: This journal, 1913, xxxi, p. 376.

and 0.6 c.c., or slightly less, at the same rate into a dog, there invariably resulted a fall in blood pressure and an increase in the depth of respiration. In some cases the same results were obtained with larger doses. Before this increase there usually was a slight shallowness in the depth of respiration occurring almost immediately after the introduction of the solution. In some experiments, however, the increase in the depth was not preceded by shallowness.

The Relation of the Depth of Respiration to the Rise in Blood Pressure

In general 0.5 c.c., or more, of a 1:100,000 solution of adrenalin njected into the circulatory system of a cat at a uniform rate



A B

FIGURE 1. Cat A. In this and all following records the upper curve indicates the blood pressure; the middle curve the contractions of the diaphragm; and the lower line the time in half-minutes. At *a*, introduction of 0.3 cc. of 1: 100,000 adrenalin solution; at *b*, introduction of 0.6 cc. of 1: 100,000 adrenalin solution.

(0.2 c.c. per second) or 1.6 c.c., or more into a dog, at the same rate, produce a rise in blood pressure, and within limits, an increase in the depth of respiration. This increase in the depth of respiration, in a given animal, as well as the rise in blood pressure is proportional to the doses of adrenalin given, Figs. 1 and 2. The increases are nearly always preceded by a shallowness. The increase may be as much as 35 per cent (Fig. 3).

On the other hand, as Oliver and Schäfer[1] reported, if very large doses, as 0.3 c.c. of a 1:1000 solution of adrenalin or more, are

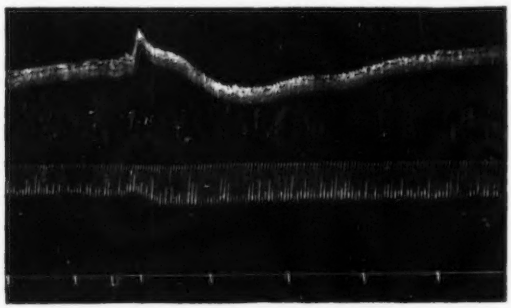


FIGURE 2. Cat A. Introduction of 1.2 cc. of 1:100,000 adrenalin solution.

injected into cats or dogs, the respiratory mechanism always responds by marked shallowness in breathing. For these large

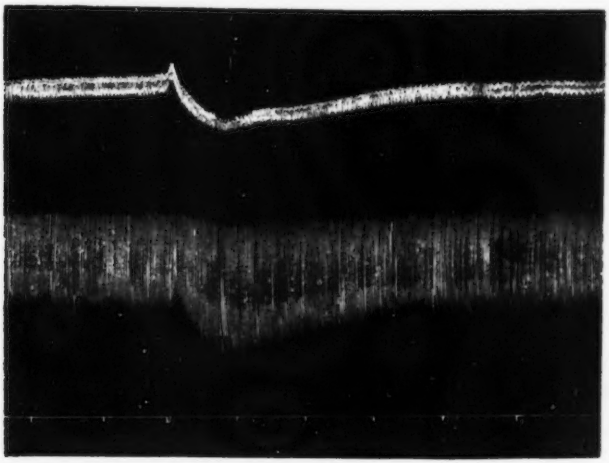


FIGURE. 3. Cat. Introduction of 0.35 cc. of 1:50,000 adrenalin solution.

doses, the decrease within limits, is in direct proportion to the dosage given and to the rise in blood pressure. In most cases the

[1] OLIVER and SCHÄFER: *loc. cit.*

shallowness was followed, as usual, by an increase in depth. In a few cases the respiration simply returned to normal.

Discussion of Results

The effect of adrenalin on the respiratory system seems to be due almost entirely to a central effect. Our results show that physiological doses have a stimulating effect on the center. Very large doses on the other hand have an inhibitory action on the respirafory center. These effects on respiration occur no matter whether the blood pressure is high or low. They also take place whether the vagi are intact or cut. By repeated doses the center becomes fatigued and the response is lessened. This is particularly true with large injections. When the breathing was irregular in some animals, the introduction of small doses of adrenalin made it regular.

Could the Increase in the Depth of Respiration be due in part to Direct Stimulation of the Diaphragm Muscle?

Considerable evidence has been brought forward to show that adrenalin does stimulate skeletal muscle to greater activity. Oliver and Schäfer injected adrenal extract into a frog and found that the excised muscle registered a curve of contraction about 33 per cent higher and 60 per cent longer than the corresponding muscle not subjected to the action of the extract.[1] Dessy and Grandis obtained a beneficial effect when adrenal extract was applied to fatigued muscle of a salamander.[2] Similar results were obtained by Panella.[3] Recently evidence was brought forth by Cannon and Nice,[4] and confirmed by Gruber,[5] to show that adrenalin indirectly improves the contraction of intact skeletal muscle in the cat by increasing the circulation through the muscle. Gruber's experiments show that the increase occurs only above a critical point in blood pressure, 90 to 100 mm. Hg. or above.

[1] Oliver and Schäfer: *loc. cit.*

[2] Dessy and Grandis: Archives italiennes de biologie, 1904, xli, p. 231.

[3] Panella: Archives italiennes de biologie, 1907, xlviii, p. 462.

[4] Cannon and Nice: This journal, 1913, xxxii, p. 44.

[5] Gruber: This journal, 1913, xxxii, p. 221.

We have been unable to find any increase in the contraction of isolated strips of diaphragm muscle, when the strip contracting in Ringer's solution in response to electrical stimuli was subjected to adrenalin.

Summary

1. The effect of adrenalin on respiration occurs synchronously with that on the circulatory system.

2. Doses of adrenalin which cause a fall in blood pressure elicit an increase in the depth of respiration. This increase may or may not be preceded by a shallowness.

3. Within limits, doses of adrenalin which produce a rise in blood pressure cause an increase in the depth of respiration. This increase, again, may or may not be preceded by a shallowness. The increase is proportional to the rise in blood pressure and to the amount of adrenalin given.

4. Excessive doses, as Oliver and Schäfer, and others have shown, produce a marked shallowness in breathing. Within limits, the shallowness is proportional to the effect on blood pressure and to the amount of adrenalin given.

FACTORS AFFECTING THE COAGULATION TIME OF BLOOD

V. The Effects of Hemorrhage Before and After Exclusion of Abdominal Circulation, Adrenals, or Intestines

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Received for publication April 30, 1914

IN 1772 Hewson noted that in an animal bleeding to death the latest blood clotted more quickly than the earliest.[1] This was confirmed in 1842 by Nasse,[2] and in 1857 by Brücke.[3] Again in 1877 confirmation of the original observation was reported by Cohnheim in these striking words: "In a dog bled to death by the removal of blood from a vein in successive portions, the last portions often coagulate almost instantaneously."[4]

In 1901 Milian noted that in a hemorrhage following capillary puncture, i.e., finger or ear, the later drops had a progressively shorter clotting time till the latest, which as got by pressure had a quarter of the time of the earliest. From this he inferred that the shortening was due to a local influx of tissue juices.[5] Arloing promptly pointed out that in a hemorrhage following venous puncture there was the same progressive shortening, although there could be no local influx of tissue juices.[6]

Again in 1904 von Weismayr remarked the shortening after

[1] HEWSON: An experimental inquiry into the properties of the blood, Experiment xxi, London, 1772, p. 69.

[2] WAGNER: Handwörterbuch der Physiologie, Braunschweig, 1842, i, p. 75.

[3] BRÜCKE: Archiv für pathologische Anatomie und Physiologie und für klinische Medizin, 1857, xii, p. 100.

[4] COHNHEIM: Allgemeine Pathologie, 1877, p. 325; or Cohnheim's General Pathology, translated by McKee, London, 1889, p. 403.

[5] MILIAN: Comptes rendus Société de Biologie, 1901, liii, pp. 556, 576.

[6] ARLOING: *ibid.*, p. 675.

hemorrhage.[1] And in 1909, Hartmann reported that the more bloody operations were generally but not invariably followed by decreased coagulation time. As to the causative factor he was unwilling to choose among diminished O_2, augmented CO_2, augmented fibrin ferment, and augmented flow of tissue thrombokinase proportionate to the size of the wound. Suddenness of the hemorrhage, however, he did define as essential to the decreased time, since he found like Schwab no decrease during the gradual exsanguination observed in myomatous women.[2] Two exceptional increases in such exsanguinated patients Hartmann viewed as due to hydration of the blood from the intestine, and in support of this view he cited[3] Terroine's repeated hemorrhages, followed by saline injections, which showed a primary decrease then a marked increase even to incoagulability.[4]

Later in 1909 von den Velden cited approvingly Nasse, Brücke, and their inference as to the independence of coagulation time and fibrin content.[5] Neither in them nor in Cohnheim nor in Hartmann could he find an adequate explanation, however; so he offered one reminiscent of them but in fact developed as an analogy to the explanation he had previously given for decreased clotting time after administration of halogen salts (bromides or chlorides). The decrease that he noticed after hemorrhage followed a loss of 19% of the circulating blood in rabbits, 8% in man. Incidentally this decrease was more marked in blood taken from veins than in that taken from capillaries. The explanation that he offered was Magnus' observation that small hemorrhages, i.e., up to 8 per cent, were followed by a thickening of the blood as measured by greater specific gravity,[6] and he gave confirming observations to show that this greater

[1] SCHRÖDER und BLUMENFELD: Handbuch der Therapie der chronischer Lungenschwindsucht, Leipzig, 1904, p. 328.

[2] SCHWAB: Münchener medizinische Wochenschrift, 1906, liii, p. 2520; and 1907, liv, p. 176.

[3] HARTMANN: Münchener medizinische Wochenschrift, 1909, lvi, p. 796.

[4] TERROINE: Comptes rendus Société de Biologie, 1907, lxii, p. 143.

[5] R. VON DEN VELDEN: Archiv für experimentelle Pathologie und Pharmakologie, 1909, lxi, pp. 37, 44.

[6] MAGNUS: Archiv für experimentelle Pathologie und Pharmakologie, 1900, xliv, p. 104.

specific gravity occurred not only after but even during the hemorrhage. This thickening was followed by a secondary hydremia (thinning) with greater rapidity in proportion to the suddenness or size of the hemorrhage, as was shown by Zimmermann [1] and often since. The factor underlying this secondary hydremia was influx of tissue juices, as shown by Regeczy in 1885.[2] Hence the analogy between von den Velden's explanation of the decreased clotting time accompanying this hydremia, and his earlier explanation of the decrease accompanying osmotic hydremia (produced by a few c.c. of 10 per cent sodium chloride intravenously).[3] The common factor in these two analogous hydremias he accordingly assumed to be that coagulating substance (Thrombokinase, zymoplastische Substanz) long recognized as present in all tissue juices. He admitted that his explanation of decreased time as due to augmented thrombokinase was apparently inconsistent with Nasse's and Brücke's independent evidence of a decrease accompanied by diminished fibrin, but he considered them reconcilable.[4]

An attempt to throw light on the problem from another angle, namely localization of the factors in coagulation, led to the observations recorded below as to the effects of hemorrhage before and after exclusion of the abdominal circulation, of the adrenals, and of the intestines. The blood of 27 cats was studied by the method described previously [5] in this series. The size of the hemorrhages produced is stated as a percentage of the normal circulating blood, after that has been estimated as 8 per cent of the body weight. Secondary hemorrhages are stated similarly as percentages of the original blood, not as percentages of the blood left in circulation after the previous hemorrhage.

[1] ZIMMERMANN: Archiv für physiologische Heilkunde, 1846, v, p. 349.

[2] REGECZY: Archiv für die gesammte Physiologie, 1885, xxxvii, p. 73.

[3] R. VON DEN VELDEN: Deutsche medizinische Wochenschrift, 1909, xxxv, p. 197; Verhandlungen des Kongresses für innere Medizin, 1909, xxvi, p. 155.

[4] R. VON DEN VELDEN: Archiv für experimentelle Pathologie und Pharmakologie, 1909, lxi, p. 42.

[5] CANNON and MENDENHALL: This journal, 1914, xxxiii, p. 225.

Hemorrhage Decreases Clotting Time

The results arranged in Table I support Hartmann's idea that in order to hasten clotting hemorrhage must be moderately sudden or severe. This seems to mean about 13 per cent of the estimated body blood; *e.g.*, 7 per cent makes no change, but a second 7 per cent halves clotting time, and after it has returned to normal it is plainly decreased by a third 7 per cent (Dec. 4); and in other hemorrhages of 13 per cent and 12 per cent at the start the decrease is plain (Dec. 5 and Feb. 28). A second hemorrhage, in other words, either may produce a decrease after an initial hemorrhage has failed (Dec. 4), or may produce a further decrease (Dec. 5).

Further details for the experiments summarized in Table I, page 336, are given in Fig. 1 for the more typical results, and for the less typical in protocols (Dec. 4 and Feb. 28).

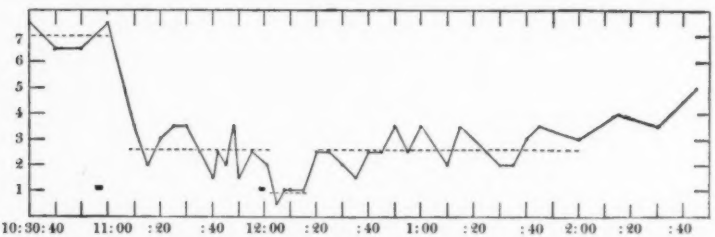

Figure 1. Record showing shortening of coagulation time after a hemorrhage (13 per cent of the blood) at 10:59, and after a second hemorrhage (10 per cent of the blood) at 11:59. The dotted lines in this and later figures indicate the averages for the time they cover.

Protocol (Dec. 4)

A cat weighing 3.3 k. was etherized at 9:20 A.M., at 10:15 decerebrated and given artificial respiration, at 10:55 the operation of preparing the femoral and carotid arteries was completed, an interval was left to allow a return to normal, after which the blood drawn at the moments stated showed the following clotting times:

11: 26–	1.0 minutes	12: 00–	2.5 minutes
11: 29–	1.5 "	12: 02–	1.0 (?) "
11: 35–	1.0 "	12: 10–	2.5 "
11: 41–	0.5 "	12: 15–	3.5 "
11: 46–	3.0 "	12: 20–	3.0 "
11: 52–	2.5 "	12: 25–	3.0 "

TABLE I

HEMORRHAGE DECREASES CLOTTING TIME (C.T.)

(In this and in later tables read from left to right along one line, then along the next line. This procedure will in many cases show on the next line a new clotting time without any new hemorrhage. This is so because a new line is used wherever the average clotting time for any group of tests varies markedly from the preceding average; e.g., On Dec. 4, the second hemorrhage of 7 per cent was followed by an interval of 2 minutes; in the next 7 minutes 3 samples were taken to test, giving an average of 1.5 minutes. Marked variation from this level is seen during the next 10 minutes by 3 samples whose average time was 3.5 minutes; hence a new line is used. Anomalous figures like 3.5 a are commented on in footnotes to each table.)

Date	C.T. before hemorrhage (Control C.T.)			Hemorrhage per cent	C.T. after hemorrhage			
	Duration of tests, minutes	No. of tests	Av. C.T. minutes		Interval minutes	Duration of tests, minutes	No. of tests	Av. C.T. minutes
Dec. 4 (Details in protocol)	15	4	1.0					
	24	5	2.3					
	10	3	3.2	7	1	10	3	3.2
				7	2	7	3	1.5
						10	3	3.5 a
				7	1	20	6	2.7
Dec. 5 (Details in Fig. 1)	30	4	7.0	13	11	51	13	2.6
				10	6	10	4	0.9
						75	16	2.6
						45	4	3.9
Dec. 16	15	4	3.0	12	1	45	8	4.5 b
				17	7	55	9	5.7
Feb. 28 (Details in protocol)	3	2	3.5					
	42	7	5.5	12	5	60	12	4.3
						60	3	5.5
						50	4	8.4a

(*a*) Secondary Rise: In many experiments the decreased coagulation time rises again not only toward normal as expected, but above. This secondary rise appears due in some cases to reaction in excess, so often remarked in biological processes when opposing factors are at work; and due in other cases to a curious progressive antemortem rise.

(*b*) Anomaly: Hemorrhage increased clotting time. This cat had been in the animal house for only three days and had been excited whenever approached. The coagulating factors roused by excitement may therefore have been exhausted (Cf. Cannon and Mendenhall: This journal, 1914, xxxiv, p. 249), so that hemorrhage was without effect.

12: 28 to 12: 29 Bled 20 c.c. from left carotid = hemorrhage of 7 per cent of body's blood.

12: 30– 3.5 minutes
12: 35– 3.0 "
12: 40– 3.0 "

12: 48 to 12: 49 Second hemorrhage 7 per cent.

12: 50– 2.0 minutes
12: 55– 1.0 "
12: 57– 1.5 "
1: 00– 3.5 minutes
1: 05– 3.5 "
1: 10– 3.5 "

1: 18 to 1: 19 Third hemorrhage 7 per cent.

1: 20– 3.5 minutes
1: 25– 2.0 "
1: 30– 2.5 "
1: 35– 3.0 "
1: 40– 2.5 " (Blood dark)
1: 45– 5.0 " (Blood dark; heart stopped)

PROTOCOL (Feb. 28)

A cat weighing 2.3 k. was etherized at 8: 00 A.M., at 8: 40 decapitated and given artificial respiration. At 10: 43 preparation of the femoral artery was completed, after which the clotting times were:

10: 45– 3.0 minutes
10: 48– 4.0 "
10: 54– 6.5 "
11: 05– 6.0 "
11: 12– 5.5 "
11: 20– 5.0 minutes
11: 25– 4.0 (?) "
11: 30– 6.0 "
11: 36– 5.5 "

11: 48 to 11: 50 Bled from femoral artery till muscular spasms = 23 c.c. = hemorrhage of 12 per cent.

11: 55– 4.5 minutes
12: 00– 4.0 "
12: 04– 4.5 "
12: 10– 4.5 "
12: 15– 4.0 "
12: 19– 3.5 "
12: 23– 4.5 minutes
12: 28– 5.5 "
12: 35– 4.5 "
12: 40– 4.0 "
12: 44– 4.0 "
12: 50– 4.0 "

12: 35 to 1: 35 Interval for lunch.

1: 37– 5.0 minutes
1: 42– 5.0 "
1: 50– 6.5 "
2: 00– 7.0 minutes
2: 15– 6.5 " (Blood dark)
2: 25– 10.0 " (Blood dark)
2: 40– 10.0 " (Blood dark; animal killed)

Where now can we localize the clotting factor or factors stimulated by hemorrhage?

Hemorrhage Fails to Decrease Clotting Time after Exclusion of the Abdominal Circulation

The earliest evidence that exclusion of the abdominal circulation increases coagulation time was given in 1886 by Stolnikow when he noted that blood circulating through heart and lungs did not coagulate in his apparatus as might be expected. He assigned as cause the fact that "the blood spent at most 20 seconds in the apparatus, after which it again was given over to the restorative influence of the vessel wall."[1] Pawlow in 1887 with similar technique noted further that the loss was gradual; i.e., blood tested when the experiment had been going on for only 15 minutes coagulated after a time, whereas blood "taken after a rather long experiment into a glass vessel showed no clot though kept till beginning decomposition." He inferred that the lungs produced some anti-clotting factor.[2]

Bohr in 1888 accepted Pawlow's experiments and inference, and supported them by shutting off the circulation at the diaphragm in two dogs and finding after about 15 minutes that carotid blood did not coagulate for twenty-four hours.[3]

In 1892 Lilienfeld thought Bohr's experiments so fundamental that he repeated them six times, and found that the time decreased twice and remained unchanged four times.[4] Prolongation of his six experiments might well have shown the more usual increase, to which the fall is only a prelude and that only sometimes (Table II *c*, March 9 and 11). His six findings are therefore quite reconcilable with ours, although we too must admit ignorance of the factor underlying that primary fall.

In 1895 Contejean objected that "the result of Bohr's experiments is hard to accord with the fact that blood from the subhepatic veins is normally almost incoagulable." But he gave no evidence. Furthermore, he repeated Bohr's experiments on dogs

[1] Stolnikow: Archiv für Physiologie, 1886, p. 10.
[2] Pawlow: Archiv für Physiologie, 1887, p. 459.
[3] Bohr: Centralblatt für Physiologie, 1888, ii, p. 263.
[4] Lilienfeld: Archiv für Physiologie, 1892, p. 152.

and cats without success . . . "the blood always remained coagulable" . . . "the blood coagulated without considerable delay." But in no instance did he give control evidence of clotting time prior to the exclusion of the abdominal circulation; and in only four instances did he give the time prior to routine experimental peptone infusions (which of course render all subsequent clotting times incomparable to Bohr's); these four were 6, 7, 14, and 17 minutes, which despite the lack of control do suggest some delay.[1] In general Contejean's effort was to "attribute to liver or intestine a preponderant part in producing the anticoagulating factor" which was active after peptone infusion, but he need not therefore have denied to liver and intestine a part in producing the normal coagulating factor. Several organs are already known to produce two different physiological factors.

In 1905 Nolf pointed out that although Doyon and Kareff were able several times to observe complete incoagulability they were obliged to sacrifice many animals. Hence he concluded that liver excision was not necessarily followed by complete incoagulability. In fact he added on the basis of numerous experiments of his own the conclusion that apart from special circumstances the incoagulability was not observed within the two hours that the dogs lived after liver excision. At the same time he admitted: "Increase of clotting time was usually noticed in the samples of blood taken after extirpation, but the clot looked normal and retracted strongly." This increase was accentuated if the operation was preceded by copious meat diet or if accompanied by portal stasis or if followed by hemorrhage. Like Pawlow he thought the increase gradual, and on this basis explained his failure to obtain the entire incoagulability previously observed by Doyon and Kareff.[2]

Although Nolf thought operative exclusion much more precise than toxic exclusion, he gave references to the accordant observations of many men who found either diminished fibrin or delayed coagulation after toxic liver injury, as by phosphorus. More extended observations of such effects were made in 1911 by Whipple and Hurwitz.[3]

[1] Contejean: Archives de physiologie, 1895, xxvii, pp. 248, 251.

[2] Nolf: Archives internationales de physiologie, 1905–06, iii, pp. 1, 7, 8.

[3] Whipple and Hurwitz: Journal of experimental medicine, 1911, xiii, p. 136.

The results arranged in Table II support (1) the inference of several of the above investigators that exclusion of the abdominal circulation excludes the clotting factor or factors and therefore

TABLE II

(1) Exclusion of the Abdominal Circulation (E) Increases Clotting Time (C.T.)
(2) Subsequent Hemorrhage No Longer Decreases Clotting Time

Date	C.T. before exclusion			Exclusion by tying vessels above diaphragm (E)	C.T. after exclusion				Hemorrhage per cent	C.T. after exclusion + hemorrhage			
	Duration of tests, min.	No. of tests	Av. C.T. min.		Interval, min.	Duration of tests, min.	No. of tests	Av. C.T. min.		Interval, min.	Duration of tests, min.	No. of tests	Av. C.T. min.
Dec. 13 (Details in Fig. 2)	15	4	4.2	E	2	55	10	5.1	5	2	56	10	5.3
									5	7	20	3	8.7
										12	24	5	5.7
Mar. 9	23	4	6.5		8	11	1	7.5					
						8	2	6.5					
				E		11	2	5.0c					
						57	4	8.2					
Mar. 11 (Details in protocol)	59	7	6.6	E	2	75	8	7.7	11	1	14	3	7.8

(*c*) This one instance of secondary brief decrease remains to be explained. Possibly as Pawlow suggested "if one compares . . . the coagulation effect of thymus . . . extract discovered by Wooldridge, one must suspect that the condition usually present in the blood should be regarded as a resultant of several mutually opposed factors arising from different organs." One of these factors is without doubt the blood platelets whose "importance in coagulation has been recognized since the work of Bizzozero and Hayem about 1880" (Lee and Vincent: Archives of internal medicine, 1914, xiii, p. 404), and whose origin has been localized by Wright in the giant cells of the bone marrow (Wright: Journal of morphology, 1910, xxi, p. 270).

increases clotting time; and also support (2) Nolf in that hemorrhage subsequent to exclusion no longer decreases the time as in animals with normal circulation, and may even accentuate the

increase previously produced by exclusion. The method here used of excluding abdominal circulation was compression (clamp or ligature) of aorta and cava just above the diaphragm, making a more completely "anterior animal" than that of Stolnikow, Pawlow, Bohr, Lilienfeld, and Contejean; though less completely anterior than that of Whipple.

Further details of the experiments summarized in Table II are given in Fig. 2, for the more typical results, and for the less typical in a protocol (Mar. 11).

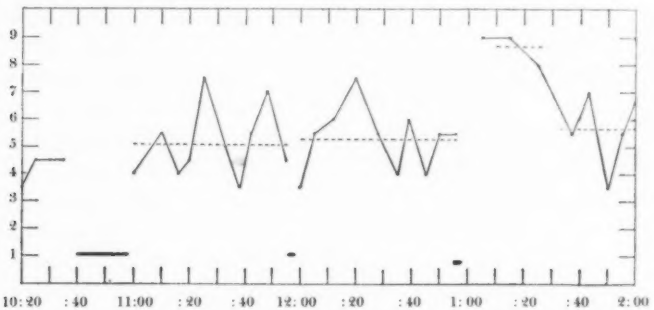

FIGURE 2. Record showing absence of rapid clotting after hemorrhage, when the circulation is confined anterior to the diaphragm. From 10:40 to 10:58 the operation of tying the aorta and inferior cava above diaphragm was performed. At 11:58 5% of the blood was drawn, and at 12:58 5% again, each time with resulting respiratory distress.

PROTOCOL (Mar. 11)

A cat weighing 2.5 k. was etherized at 8:55 A.M., at 9:15 decerebrated, at 9:25 artificial respiration. Hemostats were clamped on aorta and cava just above diaphragm, removed, applied again, removed again. While they were on the clotting time was lengthened, and shortened again after their removal. The actual figures varied much, apparently because of the shock caused by the procedure. Hence the figures are not reproduced, except for the latter part of the experiment, when the aorta and cava were not clamped but ligated (at 2:31 P.M.).

2:33– 7.5 minutes	3:23– 7.5 minutes
2:42– 6.0 "	3:31– 8.0 "
2:48– 8.5 "	3:40– 7.5 "
3:12– 8.0 "	3:48– 8.5 "

4: 11 to 4: 12 Drew 11 c.c. from carotid = 11 per cent of blood circulating in this anterior animal.

4: 13– 8.0 minutes	4: 27– 9.5 minutes
4: 21– 6.0 "	4: 37 (No blood obtainable; dead)

Hemorrhage Decreases Clotting Time after Removal of the Adrenal Glands

Having localized the clotting factor or factors in the abdomen we shall now attempt to make the localization more definite. That the adrenal glands may play a part in the clotting which follows hemorrhage was suggested by the observations that augmented adrenalin percentage in the blood hastens coagulation,[1] and that just such hyperadrenalinemia follows strong sensory stimulation,[2] which is the usual accompaniment of trauma (accidental, military, surgical) and its attendant hemorrhage.

In 1911 Trendelenburg stated that when the blood pressure was diminished by vigorous hemorrhage (7–17 per cent), the absolute amount of adrenalin secreted was maintained, i.e., there was an absolute decrease but a percentage increase of adrenalin in the blood.[3] On these facts he denied "regulation of diminished blood pressure by adrenal hypersecretion." Still his evidence of adrenal hypersecretion after hemorrhage agrees with our experience of faster clotting after hemorrhage. Incidentally it may here be noted that his denomination of 7–17 per cent as a vigorous hemorrhage accords remarkably with our experience that 7–14 per cent was the maximum possible, varying according to the animal, without producing extreme air-hunger and muscular spasms.

The question now arises, are the adrenals essential to the more rapid clotting after hemorrhage? The figures presented in Table III show that even after removal of the adrenals, hemorrhage decreases clotting time. In two instances (Feb. 18 and Mar. 2) it was noted that removal of the adrenals was followed by faster clotting — a result probably due to expression of adrenalin,[4]

[1] Cannon and Gray: This journal, 1914, xxxiv, p. 232.

[2] Cannon and Mendenhall: This journal, 1914, xxxiv, p. 251.

[3] Trendelenburg: Zeitschrift für Biologie, 1911–12, lvii, p. 98.

[4] Stewart: Journal of experimental medicine, 1912, xv, p. 547; and Hoskins and McPeek: Journal of the American Medical Association, 1913, lx, p. 1778.

which cannot be wholly obviated by even the most careful avoidance of massage (by gentle handling; and by tying off the lumbo-adrenal vein at its entrance to the gland and at its exit, as well as the gland pedicle with its vessels). In neither of these cases did the clotting process after the removal become slower than before. Thus the observations here recorded show that the adrenals are not the sole nor even the major factor, because after their removal coagulation time is not lengthened.

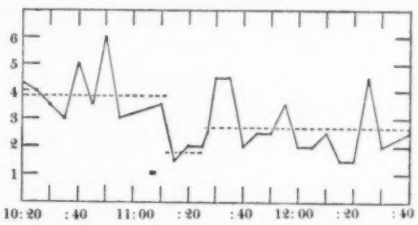

FIGURE 3. Records showing shortening of coagulation time after hemorrhage (13 per cent of the blood) at 11:08, though the adrenal glands had been previously (8:55–9:05) removed.

Further details for the experiments summarized in Table III, page 344, are given in Fig. 3 for the more typical results, and for the less typical in protocols (Jan. 7 and Mar. 2).

PROTOCOL (Jan. 7)

A cat weighing 2.4 k. was etherized without a struggle at 2:30 P.M., at 3:05 the adrenals were tied off (afferent and efferent lumbo-adrenal vein and also the pedicle), at 3:20 animal decerebrated.

3:58– 7.0 minutes
4:05– 4.0 "
4:10– 3.0 "
4:14– 4.0 minutes
4:19– 4.5 "

4:26 Pithed cord through orbit. Artificial respiration.

4:28– 3.5 minutes
4:33– 6.0 "
4:40– 4.0 minutes
4:51– 4.5 "

5:03 to 5:05 Bled till air-hunger = 35 c.c. = 18 per cent of estimated blood volume.

5:06– 3.0 minutes
5:15– 4.0 "
5:20– 2.5 "
5:25– 2.5 "
5:29– 6.0 "
5:35– 5.5 "
5:45– 9.0 minutes
6:00– 7.0 "
6:08– 7.0 "
6:15– 6.0 "
6:21 (No blood obtainable; dead)

TABLE III

HEMORRHAGE AFTER REMOVAL OF THE ADRENAL GLANDS (R) DECREASES CLOTTING TIME (C.T.)

Date	Removal of adrenals (R)	C.T. before hemorrhage				Hemorrhage per cent	C.T. after hemorrhage			
		Interval, min.	Duration of tests, min.	No. of tests	Av. C.T., min.		Interval, min.	Duration of tests, min.	No. of tests	Av. C.T., min.
Dec. 6 (Details in Fig. 3)	R	65	55	10	3.8	13	7	10	3	1.8
								73	15	2.7a
Dec. 17	R	30	35	5	5.3					
			15	3	4.0	8	7	48	7	5.5d
						6	5	35	7	3.7
Dec. 18	R	25	85	14	3.0	20	1	10	2	2.0
Jan. 7 (Details in protocol)	R	53	7	1	7.0					
			14	4	3.9					
			23	4	4.5	18	1	19	4	3.0
								46	6	6.7a
Feb. 18	R	5	5	2	4.0					
			240	24	5.4	5	1	25	4	8.0e
Mar. 2 (Details in protocol)	R	5	27	5	5.7					
			64	7	6.7	14	5	9	1	8.5f
								20	4	4.9
								65	9	6.6a
						9	5	24	5	7.0e

(*a*) cf. Table I.

(*d*) This 5.5 though higher than 4.0 is so little higher than 5.3 or than their average 4.8 that it very likely lies within the limits of error and means only that the small hemorrhage had no more effect than on Dec. 4, Table I. Note that, as on that date, a second hemorrhage produces the expected decrease.

(*e*) This increase may mean either that the hemorrhage had no more effect than on Dec. 4, Table I, so that the preceding secondary rise (e.g., on Feb. 18 secondary to the primary fall 4.0. The control average clotting time before removal of the adrenals was 6.2 minutes) simply continued uninterrupted; or it may mean an anomaly yet to be explained.

(*f*) This increase may mean either of the two possibilities under *e* (the average clotting time before removal of the adrenals was 6.9 minutes); or thirdly, being a single test, may mean an artefact due to leakage from the water bath through the rubber connection which was somewhat old and was in this case found to have become suddenly loose, probably due to too long rinsing in the beaker of ether.

PROTOCOL (Mar. 2)

A cat weighing 2.1 k. was etherized at 9: 55 A.M., without a struggle, at 10: 20 decerebrated, at 10: 35 preparation of femoral artery completed.

10: 40– 5.0 minutes
10: 47– 5.0 "
11: 00– 5.5 "
11: 10– 7.0 "
11: 20– 6.5 minutes
11: 30– 7.0 "
11: 40– 7.0 "

11: 45 to 12: 10 Removal of adrenals.

12: 15– 7.0 minutes
12: 23– 6.5 "
12: 30– 5.5 "
12: 36– 5.0 "
12: 42– 4.5 "
12: 55– 6.0 "
1: 02– 7.0 minutes
1: 26– 7.5 "
1: 34– 7.0 "
1: 42– 6.5 "
1: 50– 7.0 "
1: 59– 6.0 "

2: 09 to 2: 11 Bled from femoral artery till muscular twitching (not till spasms) = 24 c.c. = hemorrhage 14 per cent of circulating blood.

2: 16– 8.5 minutes
2: 25– 4.5 "
2: 30– 5.5 "
2: 38– 4.0 "
2: 45– 5.5 "
2: 52– 7.5 "
3: 01– 6.5 "
3: 08– 8.0 minutes
3: 22– 7.5 "
3: 30– 5.0 "
3: 40– 6.0 "
3: 47– 5.5 "
3: 57– 6.0 "
4: 09– 7.5 "

4: 21 to 4: 22 Bled 15 c.c., no spasms resulting nor air-hunger = hemorrhage 9 per cent.

4: 25– 7.0 minutes
4: 32– 7.0 "
4: 40– 6.0 "
4: 49– 7.0 minutes
4: 57– 8.0 "

5: 15 Pithed cervical cord through orbit; artificial respiration.

5: 20– 11.0 minutes
5: 32– 6.5 "
5: 40– 5.5 "
5: 46– 4.5 minutes (Blood dark; pressure low)
5: 51– 7.5 minutes (Blood dark; pressure low)
6: 00 (No pressure; dead)

Hemorrahge Decreases Clotting Time after Exclusion of the Intestines

In 1899 Mathews[1] published an experiment in which tying of the superior and inferior mesenteric arteries and the mesenteric vein in a cat was followed by marked increase of the coagulation time. Although Brücke stated that the shortening after hemorrhage was not paralleled by abundance of fibrin, and although similarly Mathews showed convincingly that the lengthening after exclusion of the intestine was not paralleled by absence of fibrinogen, still it is interesting to observe that the long-standing belief in the parallelism between the rate of clotting and the quantity of fibrinogen is supported by the agreement of the experiments here reported with a note by Goodpasture earlier this year that ligature of the intestine or cutting off half the blood to the liver caused a marked delay in fibrinogen reproduction after complete defibrination.[2]

Goodpasture quoted Bohr's and Mathews' evidence for incoagulability after exclusion of intestine, and in opposition gave only the general statement that in his experiments "specimens taken at frequent intervals during the perfusion have nearly always clotted within the normal time, one to seven minutes." It seems as if with less liberality as to the range of normal time he might well have found a significant variation in coagulation time, whether increase or decrease.

The results presented in Table IV support (1) both Bohr and Mathews by showing that exclusion of the small and half the large intestine increases clotting time. This increase is preceded by no such primary decrease as occurred immediately after adrenalectomy, and this is as might be anticipated from the fact that the circulation through the intestines can be easily cut off without massage of the adrenals or other viscera. The intestine, therefore, like the adrenal gland is a factor in clotting. But, again like the adrenal, it is not the sole nor even the major factor, because, as the Table further shows, hemorrhage after exclusion of the intestines can still cause a decrease in clotting time.

[1] Mathews: This journal, 1899, iii, p. 79.

[2] Goodpasture: This journal, 1914, xxxiii, p. 85.

TABLE IV

(1) EXCISION OF THE SMALL AND HALF THE LARGE INTESTINES (E) USUALLY INCREASES CLOTTING TIME (C.T.)

(2) SUBSEQUENT HEMORRHAGE DECREASES CLOTTING TIME

Date	C.T. before excision			Excision	C.T. after excision				Hemorrhage per cent	C.T. after excision + hemorrhage			
	Duration of tests, min.	No. of tests	Av. C.T., min.		Interval, min.	Duration of tests, min.	No. of tests	Av. C.T., min.		Interval, min.	Duration of tests, min.	No. of tests	Av. C.T., min.
Dec. 11 (Cf. protocol)				E	15	11	3	4.0	9	2	34	9	3.7
											10	3	4.5
									6	3	20	6	2.7
Jan. 8 (Cf. protocol)				E	25	10	3	4.8	13	2	17	3	6.3g
											48	7	3.9
									8	2	3	1	2.5
											9	3	5.2a
Feb. 20	58	6	10.6	E	10	11	2	12.2	?	3	20	2	7.5
Feb. 21 (Cf. Fig. 4)	35	5	7.0	E	8	37	5	8.8					
					65	10	2	9.7	10	5	36	6	5.4
Mar. 3	54	7	6.1	E	5	15	4	4.5h					
						5	2	5.7	15	5	19	4	4.9
												1	7.5a

(*a*) cf. Table I.

(*g*) Anomaly: In one case, hemorrhage after excision of the intestine caused, before the usual decrease, a marked increase of coagulation time. This primary increase may indicate that excision of the intestine removes the major coagulating factor, and that some time must elapse before the substitute factor gets to work in making the usual decrease.

(*h*) Anomaly: In one case, excision of the intestine, after tying through and injuring the pancreas instead of removing it intact, caused a decrease. Responsibility lies therefore to all appearances in some way with the pancreas, although such a conclusion appears contrary to the experiment of Mathews cited above on p. 346.

Further details for the experiments summarized in Table IV, page 347, are given in Fig. 4 for the more typical results, and for the less typical in protocols (Dec. 11 and Jan. 8).

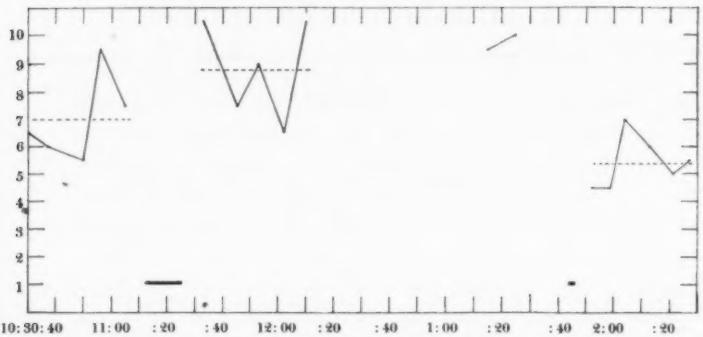

FIGURE 4. Record showing lengthening of coagulation time after removal of the small and half of the large intestine (11:12–11:25), with shortening of the coagulation time after subsequent hemorrhage (10 per cent of the blood) at 12:46.

PROTOCOL (Dec. 11)

A cat weighing 3.3 k. was etherized at 8:25 A.M.; at 8:35 vessels tied off in mesentery near intestine; at 8:45 the small and half of large intestine excised; at 8:50 preparation of the femoral artery completed; and at 8:55 the animal was decerebrated.

9:00– 4.5 minutes
9:05– 6.0 "
9:11– 5.5 "
9:17– 5.0 "
9:23– 4.0 minutes
9:28– 4.0 "
9:34– 4.0 "

9:39 to 9:40 Bled 23 c.c. = 9 per cent of total estimated blood.
9:41– 4.5 minutes (Blood dark)
9:46– 3.0 " (" ")
9:50– 2.5 " (" normal)
9:53– 5.0 "
9:58– 4.0 "
10:03– 4.5 minutes
10:08– 3.5 "
10:12– 3.0 "
10:15– 4.0 "

10:19 to 10:29 Tied off gastro-hepatic omentum except hepatic artery.
10:20 Artificial respiration.
10:30– 4.5 minutes
10:35– 4.5 "
10:40– 4.5 "

10: 52 to 10: 53 Bled 15 c.c. = 6 per cent.

10: 55–	3.0 minutes	11: 05–	3.0 minutes
10: 58–	2.0 "	11: 08–	4.5 "
11: 00–	2.0 "	11: 15–	2.0 " (Blood dark; heart stopped)

PROTOCOL (JAN. 8)

A cat weighing 3.7 k. was etherized with a marked period of excitement at 2: 20 P.M., at 2: 50 decerebrated and given artificial respiration; at 2: 55 vessels tied off in mesentery near intestine, the small and half the large intestine excised; at 3: 05 abdomen closed with clamps.

3: 20–	4.5 minutes
3: 25–	5.0 "
3: 30–	5.0 "

3: 41 to 3: 42 Bled till air-hunger = 38 c.c. = 13 per cent.

3: 43–	7.5 minutes	4: 25–	3.0 minutes
3: 50–	5.5 "	4: 35–	4.0 "
4: 00–	6.0 "	4: 40–	4.0 "
4: 07–	4.0 "	4: 45–	5.5 "
4: 15–	3.5 "	4: 55–	3.5 "

5: 01 to 5: 02 Bled till air-hunger = 24 c.c. = 8 per cent.

5: 03–	2.5 minutes	5: 15–	6.5 minutes
5: 06–	4.0 "	5: 23	(No blood obtainable; dead)
5: 10–	4.5 "		

DISCUSSION

The results presented in the foregoing pages confirm earlier observations by showing that clotting time is shortened by hemorrhage when that is sudden and of sufficient amount (at least 13 per cent of the blood volume). That this effect is due to some change in the abdominal viscera is indicated by a failure of the blood to clot faster when hemorrhage occurs after the circulation is confined anterior to the diaphragm. Previous experimentation has pointed to the adrenal glands, the intestines, and the liver, as organs in the abdomen which are concerned with clotting.

As proved by an earlier paper in this series stimulation of the adrenal glands results in more rapid clotting.[1] From Trendelenburg's evidence, previously cited, that the percentage of adrenalin in the blood is increased after hemorrhage it is probable that the faster clotting which follows bleeding is due to effects on the adrenal glands, in part. On the other hand the shortening of coagulation time by hemorrhage after the adrenal glands have been removed proves that they are not an essential factor in the phenomenon.

Several earlier investigators have studied the intestine-liver complex. In 1888 Bohr noticed that exclusion of the intestine and liver by tying splanchnic arteries produced after four hours no untoward symptoms but a blood which after withdrawal showed no clot until more than two hours had passed and even then it was abnormal, small and soft.[2] In 1899 Mathews published three experiments in which exclusion of intestine and liver by tying most of the splanchnic vessels was followed by marked increase from about 2 minutes to 30; and one experiment in which excision of the spleen and pancreas was followed by no increase. The fibrinogen content, however, did not change, and the clot became complete only after hours (as Hammarsten and Schmidt had shown to occur in solutions of fibrinogen poor in ferment), wherefore he ascribed the increase "probably to a diminution of fibrin ferment."

The experiments in which we removed the small intestine and half the large, support the evidence adduced by several previous workers that the intestines are probably an important agency in providing a factor or factors favorable to blood clotting. But they too are not essential, for after they have been thus eliminated, hemorrhage still shortens the coagulation time.

The liver is left for consideration. Its importance for coagulation is well established. In 1904 Doyon and Kareff excised the liver in a dog whose blood coagulated in 3 minutes and joined the portal to a subhepatic vein, *i.e.*, cava. After this, one specimen coagulated in 8 minutes, one in 20, and two not at all.[3] They

[1] Cannon and Mendenhall: This journal, 1914, xxxiv, p. 243.

[2] Bohr: *loc. cit.*

[3] Doyon and Kareff: Comptes rendus Société de Biologie, 1904, i, p. 612.

confirmed the observation several times.[1] In 1912 Meek found that after an Eck fistula fibrinogen regenerated more slowly than normally,[2] and similarly Goodpasture noted this year that cutting off half the blood to the liver caused a slight but noticeable reduction in the rate of fibrinogen regeneration after complete defibrination.[3] In two experiments (not here detailed) we found, like previous observers, that exclusion of the liver prolongs coagulation time.

On the basis of evidence given above it is probable that of the organs in the abdomen affecting the clotting time of blood those most directly concerned with faster coagulation after hemorrhage are the intestines and liver. Certainly the results we have obtained show that the liver is capable of producing this hastening in the absence of the intestines. Our experiments have not permitted us, however, to testify whether the liver is or is not more effective than the intestines in the shortening of coagulation time which follows rapid withdrawal of blood from the body.

Summary

I. Hemorrhage decreases clotting time, especially if moderately severe — 13 per cent of the circulating blood.

II. The most important coagulating factors may be localized in the abdomen, because: (1) exclusion of the abdominal circulation increases clotting time, and (2) hemorrhage after exclusion no longer stimulates a decrease of clotting time.

III. The adrenal glands probably favor by their secretion rapid clotting after hemorrhage, but after adrenalectomy hemorrhage still decreases the clotting time.

IV. Exclusion of the intestines usually increases clotting time, but after this exclusion hemorrhage can still decrease the clotting time.

V. The important rôle which the liver has been proved to play in coagulation indicates that it provides, perhaps in cooperation with the intestines and the adrenal glands, the necessary elements to hasten clotting after extensive bleeding.

It is a pleasure to acknowledge our indebtedness to Dr. W. B. Cannon for his advice and aid.

[1] Cited by Nolf: *loc. cit.* [2] Meek: This journal, 1912, xxx, p. 161.
[3] Goodpasture: *loc. cit.*, p. 84.